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## CORRELATION BETWEEN GRADES ON SCORES AND GRADES ON CRITICISMS IN THE JUDGING OF DAIRY PRODUCTS

WILLIAM WHITE, P. A. DOWNS, M. J. MACK, E. L. FOUTS  
AND G. M. TROUT, CH.

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The judge of dairy products is required not only to place numerical scores on the products being judged, but also to criticize them as well. Considerable proficiency is often manifest by judges in placing a numerical value or score on a product. On the other hand, the specific reason for giving the sample a certain numerical value is frequently not so readily forthcoming. The various flavors, textures, and so on, which go to make up the general quality of the sample may be rather difficult to identify and to describe as compared to the placing of a numerical value, representing a degree of quality, upon the sample.

Apparently in the judging of any dairy product such as butter, cheese, milk and ice cream, a judge must have separate abilities; first, the ability to recognize quality in a product and to place a numerical value on the product commensurate with it, and second, the ability to identify and describe the items which make up that quality. This paper presents data showing correlations between those abilities as noted in the grades of student judges.

These data were secured from the 1938 National Dairy Products Judging Contest in which twenty-three college teams of three men each competed in the judging of butter, cheese, milk, and ice cream. In this contest, 69 contestants scored and criticized seven samples each of the above products. Grades were thus available on 483 contestant-sample judgments per product.

### GRADING OF CONTESTANTS

In dairy products judging the contestant's grade is a negative grade, being in part the difference between the official score and the contestant's score, and in part, the grade, not exceeding one point per score card item, based upon the contestant's ability to describe the quality as indicated and

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Credit is due Dr. W. D. Baten, Mathematics Department, Michigan State College, for guidance in the statistical treatment of the data of this study, and to Dr. G. P. Deyoe, Department of Education, Michigan State College, for a critical reading of the manuscript and for suggestions pertaining to possible predictions of the study.

described by the official judge. Obviously, the contestant with the lower grade has the higher rank in judging ability, inasmuch as his judgment is closer to the official judgment. The compilation of a contestant-sample grade, for example, in the judging of cheese, may be illustrated as shown in table 1.

TABLE 1  
*The method by which a contestant grade is compiled*

Item	Score		Grade on score	Criticism		Grade on criticism
	Contestant	Official		Contestant	Official	
Flavor . . .	39	37	2.0	acid	bitter	1.0
Body and texture . . .	28	27	1.0	mealy	mealy	0.0
Finish . . .	15	15	0.0	.....	.....	0.0
Color . . .	10	10	0.0	.....	.....	0.0
Total . . .			3.0			1.0

Grade on sample,  $3.0 + 1.0 = 4.0$

The sum of the contestant grades on the seven samples represents his grade for that product. Naturally in this method of grading much depends upon the correctness of the official scoring of the product. To this end an official judge assisted by two coach judges does the judging for a specific product. In the 1938 contest the official judges were: butter, J. S. Edwards, assisted by coach judges E. O. Herreid and S. T. Coulter; cheese, H. L. Wilson, assisted by L. C. Thomsen and K. R. Renner; milk, C. J. Babcock, assisted by I. A. Gould and F. J. Doan; and ice cream, A. C. Dahlberg, assisted by W. H. Martin and N. E. Fabreins.

#### DISTRIBUTION OF CONTESTANT GRADES

The percentage distribution of the contestants according to total grades in the judging of butter, cheese, milk and ice cream are given in table 2. Here it will be noted that the mean grade was lowest in butter judging, being  $14.63 \pm 0.33$ ; was slightly higher in cheese and in milk judging,  $17.55 \pm 0.52$  and  $16.97 \pm 0.48$  respectively; and was  $26.90 \pm 0.51$  in ice cream judging, nearly twice that for butter.

These variations in mean values, particularly a low value in butter and a high value in ice cream, would seem to indicate that butter scoring was more nearly standardized throughout the country than ice cream scoring, or that fewer items were cut in the scoring of butter than in the scoring of ice cream, or both.

Concerning the items cut, the official scores show that 7, 12, 11, and 13 items were cut and criticized in the scoring of seven samples each of butter, cheese, milk and ice cream respectively. Hence ice cream scoring seems not only to be less standardized, but includes slightly more items cut and criti-

TABLE 2

*Percentage distribution of contestants, according to grades, in the judging of seven samples each of four dairy products, Students' National Dairy Products Judging Contest, 1938*

Class interval of grade	Percentage distribution of contestants judging			
	Butter	Cheese	Milk	Ice cream
	Per cent	Per cent	Per cent	Per cent
4.1 - 6.0	1.43			
6.1 - 8.0		4.30	1.43	4.30
8.1 - 10.0		2.87	4.30	1.43
10.1 - 12.0		25.83	14.34	11.48
12.1 - 14.0		20.09	12.92	12.92
14.1 - 16.0		15.79	7.18	11.48
16.1 - 18.0		12.48	15.79	18.22
18.1 - 20.0		8.61	18.21	11.48
20.1 - 22.0		5.74	4.30	18.66
22.1 - 24.0		1.43	7.18	2.87
24.1 - 26.0			7.18	2.87
26.1 - 28.0			2.87	2.87
28.1 - 30.0			2.87	
30.1 - 32.0				1.43
32.1 - 34.0			1.43	
34.1 - 36.0				2.87
36.1 - 38.0				4.30
38.1 - 40.0				1.43
40.1 - 42.0				1.43
42.1 - 44.0	1.43			
No. of contestants	69	69	69	69
Mean grade* for 7 samples	14.63 ± 0.33	17.55 ± 0.52	16.97 ± 0.48	26.90 ± 0.51
Mean grade per sample	2.09	2.51	2.42	3.84
Mean grade per score card item†	0.52	0.83	1.21	1.28

\* These were obtained from ungrouped data.

† Mean grade for butter for 68 contestants only.

† Package in butter, finish in cheese, and sediment in milk not subject to criticism in the students' contest; the above calculations based upon a total of 28 items for seven samples each of butter and of cheese, upon a total of 14 items for milk, and upon 21 items for ice cream.

cized, which increases the possibility of a higher contestant grade in that product.

The data in table 2 are arranged to show the distribution of contestants according to grades per product, and the averages per sample and per score card item. On a per item basis higher grades, which represent lower judging ability, were noted in the judging of milk and of ice cream than of butter and cheese. However, it must be borne in mind that a higher percentage of the items on the butter score card than on the cheese, milk, and ice cream score cards are never cut in the scoring of that product. On the other hand, these items are subject to cuts and have therefore been included in the averages. This accounts in large part for the low per item grade in butter judging.

## STANDARDIZATION OF BUTTER JUDGING IN RESPECT TO JUDGING OF BODY

Despite the assumed standardization of butter judging throughout the country, the number of contestants who persisted in cutting the body of butter, a phase of butter judging about which the modern butter judge is extremely lenient, was surprisingly high. Only one sample of butter, number 6, was cut in body by the official judges in the 1938 contest. Yet, just a single contestant of the 69 contestants cut the body of this sample only. Seventeen of the 69 contestants or 24.6 per cent, scored all the samples of butter perfect in body; 13 cut the body of only one of the seven samples, but not necessarily the same sample; 18 cut the body of two samples; 11 cut the body of three samples; 4 cut the body of four samples; and 6 cut the body of five samples. Likewise interesting are the total numbers of contestants cutting the body of specific samples of butter. These, with the official scoring, in part, are shown in table 3.

TABLE 3

*The number of contestants cutting the body of specific samples of butter*

Sample number	Number out of 69 contestants who cut the body of the various samples of butter	Official total score of butter samples	Body of butter cut by official judges, with criticism
1 .....	14	88	-
2 .....	38	93	-
3 .....	10	91	-
4 .....	17	92	-
5 .....	23	88	-
6 .....	15	89.5	+, crumbly
7 .....	7	90	-

Sample 6 was criticized by the official judges as being "crumbly." Possibly this condition was not so noticeable when the sample warmed during the four hours of scoring, as indicated by the relatively few who criticized the body of this sample. Consequently, the group of contestants scoring butter last may have considered the crumbly condition, if noted at all, as not sufficiently serious to merit a cut.

Thirty-eight of the 67 contestants, or 55 per cent, criticized the body of sample 2, which was a 93 score butter having a smooth waxy body and which was not criticized officially in any respect.

This discussion of the student scoring of the body of butter is included merely to point out that that which was thought to be so standardized may not be so standardized after all. Many data of a similar nature may be presented on other items of the same and of other products but are without the scope of this paper.

## ABILITY TO SCORE VERSUS ABILITY TO CRITICIZE DAIRY PRODUCTS

Many coaches believe that if a student can score a product with a high degree of proficiency he can also describe the product with considerable

accuracy. Likewise, many contestants who have scored the products very close to the official score have been embarrassed to learn that their total grade was much higher than anticipated, due to the added grades on criticisms. To ascertain what relationship existed between ability of student judges to score and their ability to criticize the various dairy products, the data were subjected to detailed study.

In grouping the contestants according to their proficiencies, either in scoring or in criticizing, their grades were grouped, first, according to grade on score and, second, according to grade on criticism. Lack of or presence of ability in scoring or in criticizing was assumed when the grade was above or below a certain standard which was determined by inspection of the ungrouped data.

When the contestants were thus grouped according to their abilities to score or to criticize the product, their supplementary grades on criticisms or scores, whichever the case may have been, were summarized. These groupings and mean grades are presented in tables 4 and 6. A study of the data of table 4 shows that, regardless of the product, the contestant showing

TABLE 4

*Student grades on criticizing seven samples each of butter, cheese, milk and ice cream as indicated by their proficiencies in scoring the samples*

Product	Mean grade on "criticism" when grade on "score" was		
	No. of students	Below 10.0*	Above 10.0**
Butter	34	$5.48 \pm 0.22$	$6.20 \pm 0.28$
Cheese	29	$7.36 \pm 0.37$	$5.10 \pm 0.29$
Milk	17	$7.89 \pm 0.40$	$3.13 \pm 0.25$
Ice cream	33	$17.22 \pm 0.44$	$5.59 \pm 0.31$
<hr/>			
Butter	35	$10.43 \pm 0.79$	$6.77 \pm 0.27$
Cheese	40	$14.53 \pm 0.47$	$6.72 \pm 0.24$
Milk	52	$14.46 \pm 0.43$	$4.56 \pm 0.23$
Ice cream	36	$24.27 \pm 0.44$	$6.37 \pm 0.23$

\* Below and above 7.0 in the case of butter.

\*\* Below and above 20.0 in the case of ice cream.

superiority in scoring exhibited little superiority over the poorer scorers in criticizing the samples. The difference between the grades on criticism of the two groups, except in cheese judging, was not statistically significant, as shown in table 5.

A study of the data of table 6 on the other hand shows that a contestant exhibiting ability to criticize the sample, also usually has ability to score the sample with considerable proficiency. These observations are particularly

TABLE 5

*Significance of differences in mean grades on criticisms of the low- versus the high-scoring group of contestants*

Product	Mean grades on criticism of		Difference in grades	t value for difference; statistical significance <sup>1</sup>
	Low group	High group		
Butter .....	6.20 ± 0.28	6.77 ± 0.27	0.57	1.50*
Cheese .....	5.10 ± 0.29	6.72 ± 0.24	1.62	4.25†
Milk .....	3.13 ± 0.25	4.56 ± 0.23	1.43	1.83*
Ice cream .....	5.59 ± 0.31	6.37 ± 0.23	0.78	2.02††

<sup>1</sup> Table III, Mathematical Statistics, W. D. Baten, John Wiley and Sons, N. Y.

\* Not significant.

† Highly significant.

†† Border line case.

TABLE 6

*Student grades on scoring seven samples each of butter, cheese, milk and ice cream as indicated by their proficiencies in criticizing the samples*

Product	Mean grade on "score" when grade on "criticism" was		
	No. of students	Mean grade on "criticism"	Mean grade on "score"
Butter .....	16	4.28 ± 0.21	5.93 ± 0.51
Cheese .....	26	4.25 ± 0.16	8.53 ± 0.56
Milk .....	25	2.51 ± 0.11	11.31 ± 0.73
Ice cream .....	27	4.23 ± 0.05	19.40 ± 0.03

	Above 5.0*		
	No. of students	Mean grade on "criticism"	Mean grade on "score"
Butter .....	53	7.16 ± 0.16	8.80 ± 0.58
Cheese .....	43	7.13 ± 0.17	13.31 ± 0.64
Milk .....	44	5.17 ± 0.19	13.59 ± 0.60
Ice cream .....	42	7.14 ± 0.15	21.85 ± 0.83

\* Below and above 3.5 in the case of milk.

TABLE 7

*Significance of differences in mean grades on scores of the low- versus the high-criticizing group of contestants*

Product	Mean grades on scores of		Difference in grades	t value for difference; statistical significance <sup>1</sup>
	Low group	High group		
Butter .....	5.93 ± 0.51	8.80 ± 0.58	2.87	2.33*
Cheese .....	8.53 ± 0.56	13.31 ± 0.64	4.78	5.15†
Milk .....	11.31 ± 0.73	13.59 ± 0.60	2.28	2.37*
Ice cream .....	19.40 ± 0.03	21.85 ± 0.83	2.45	2.35*

<sup>1</sup> Table III, Mathematical Statistics, W. D. Baten, John Wiley and Son, N. Y.

\* Significant.

† Highly significant.

interesting in view of the commonly accepted idea that a student who exhibits scoring ability can be relied upon to criticize the sample with accuracy. Rather the reverse seems to be true. According to these data, the student superior in criticizing the samples is likely to be superior also in scoring the samples. The differences between the mean grades on the scores of the superior and of the inferior groups in criticizing ability were statistically significant, regardless of the product, as shown in table 7. These observations would seem to be of special importance from the pedagogical standpoint in the training of student judges.

**CORRELATION BETWEEN ALL CONTESTANTS' GRADES ON SCORES AND THEIR GRADES  
ON CRITICISMS**

In view of the previously shown lack of or lesser ability of a contestant to describe a sample, although he may have scored it with proficiency, it seemed desirable to determine the coefficients of correlation between the grades on scores and the grades on criticisms for all the contestants as a single group. Accordingly, this was done on the pairs of grades per contestant per product. The correlation coefficients and their statistical significances are included in table 8.

TABLE 8

*Coefficients of correlation between grades on scores and grades on criticisms in the judging  
of dairy products*

Group	Product	Number of pairs of observations	Coefficients of correlations <sup>1</sup>
Individuals	Butter	68	0.3064*
	Cheese	69	0.8214†
	Milk	69	0.3193†
	Ice cream	69	0.4456†
	All products	275	0.2354†
Teams	Butter	23	0.8726†
	Cheese	23	0.6876†
	Milk	23	0.2641 (Not sign.)
	Ice cream	23	0.4615*
	All products	92	0.1981 (Not sign.)

<sup>1</sup> Fisher's table VA.

\* Significant at the 5 per cent level.

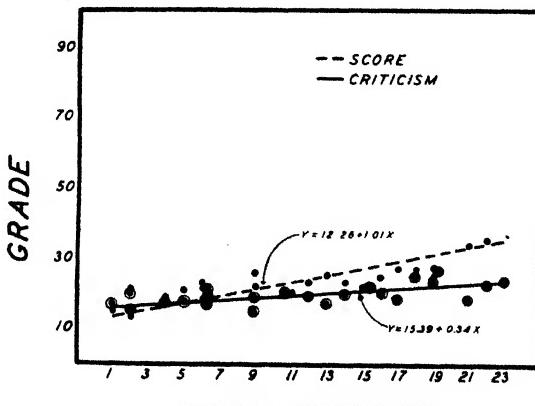
† Significant at the 1 per cent level.

Less correlation between contestant grades on scores and on criticisms was found in butter judging than in any other product. This was followed very closely by milk, then by ice cream. The greatest coefficient 0.8214, between contestant grades on scores and on criticisms was noted in cheese judging. This high coefficient between cheese grades and low coefficients between butter, milk, and ice cream grades, may be explained, in part, by the fact that the presence of or lack of quality in cheese may often be noted by the eye, and that which may be seen may be described more accurately, and,

in part, by the fact that samples identical to the three preliminary or key samples were included in the group of seven samples which were judged in the 1938 contest. Undoubtedly several students recognized one or more of these three samples and thus scored them as the official judges had scored them.

The lowest coefficient between individual grades on scores and grades on criticisms, 0.2354, was found when the correlation was calculated between the grades of all the products as a whole. The correlation between the individual grades on scores and grades on criticisms of butter was significant at the 5 per cent level, whereas those on milk, cheese, and ice cream were highly significant, as shown in table 8. Most of the coefficients of correlation shown in table 8 are statistically significant; *i.e.*, the chances are no more than 5 out of 100 that the relationships could have occurred from fluctuations in sampling.

However, it is important to interpret these coefficients from the standpoint of their value for predictive purposes. Using the highest coefficient of correlation, 0.8726, as a basis for predicting\* the grade of a team on one trait from the grade on the other trait, it would be possible to make the prediction with an accuracy which would be slightly over 50 per cent better than a guess. Taking the lowest correlation which is statistically significant, 0.2354, based on grades for all products for individuals, the prediction would be only about 3 per cent better than a sheer guess. The other coefficients, if used in a similar manner would provide predictions with degrees of effectiveness between these extremes. These may be computed by using the formula of  $\sqrt{1 - r^2}$  and the resultant figure subtracted from 1.00. For example, a correlation coefficient of 0.30, such as was found between the grades of indi-



### TEAM STANDING

FIG. 1. Lines of regression of team grades on scores and on criticisms in the judging of butter.

\* Statistics in Psychology and Education, Garrett, H. E., Longmans, Green & Co., New York City. p. 345.

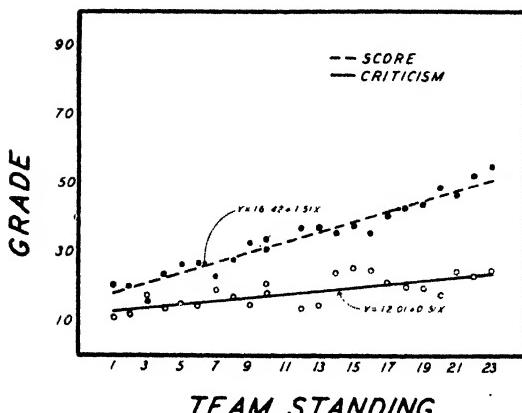


FIG. 2. Lines of regression of team grades on scores and on criticisms in the judging of cheese.

viduals in butter judging, would provide a prediction which would be about 5 per cent better than a guess.

It is doubtful, therefore, if anyone would wish to hazard a prediction based on any of the coefficients, with the possible exception of the highest. Such comparisons as these give further indication that the traits or abilities in question are not closely similar.

#### CORRELATION BETWEEN TEAM GRADES ON SCORES AND THEIR GRADES ON CRITICISMS

The correlation coefficients between team grades on scores and team grades on criticisms are also included in table 8. The observed correlations

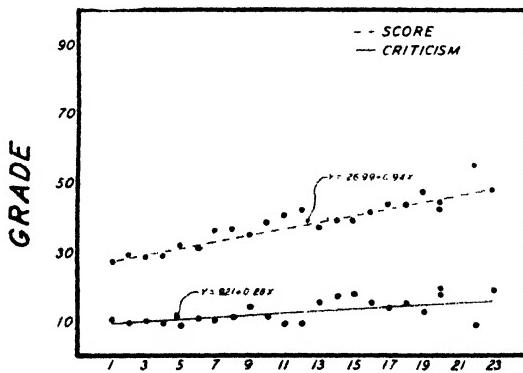
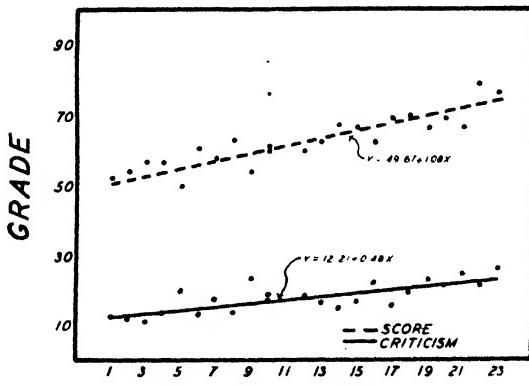


FIG. 3. Lines of regression of team grades on scores and on criticisms in the judging of milk.

between team grades on scores and on criticisms in butter and cheese judging were highly significant, that on ice cream was significant, whereas those on milk judging and on all products were not statistically significant.

The correlation coefficient between the grades on scores and on criticisms of *individuals* judging butter was 0.3064, which was significant at the 5 per cent level, whereas that of the *team* in the same respect was 0.8726, which was highly significant. Apparently the groups of individuals comprising the teams were able to score and to criticize the samples with high correlation between the grades on scores and the grades on criticisms, but individually showed so much variation that less significance could be attributed to the coefficient observed.



TEAM STANDING

FIG. 4. Lines of regression of team grades on scores and on criticisms in the judging of ice cream.

The coefficient between grades of scores and grades of criticisms of the teams in cheese judging, like that of the individuals, was highly significant. In ice cream and milk judging, especially milk, less significance could be attributed to the coefficients of the team grades than that of the individuals as a group. These observations would seem to indicate that the standard of judging for some products are fairly well fixed in the minds of the coaches while those of other products are not so well established, assuming in the coaching that equal emphasis and time had been allotted to each product. On the other hand, it is not unlikely that the flavors current to some products, milk and ice cream for example, may be more difficult to identify than those for butter and cheese. Hence, lower coefficients are observed between score and criticism grades in those products. Apparently, these lower correlations between team grades on scores and on criticisms of milk and of ice cream were more than sufficient to counterbalance the higher correlations between the pairs of grades in butter and in cheese judging, thus resulting in a non-significant coefficient of 0.1981 between the grades in all products.

The team grades on scores and on criticisms in butter, cheese, milk, ice cream and all products judging, showing lines of regression, are presented graphically in figures 1, 2, 3, 4, and 5 respectively. In all cases the lines seem to fit the data very well as can be seen by examining the figures. Likewise, the standard errors of estimate were calculated from the data of figures 1 and 2. These standard errors were found to be quite small, and thus were not included in the figures.

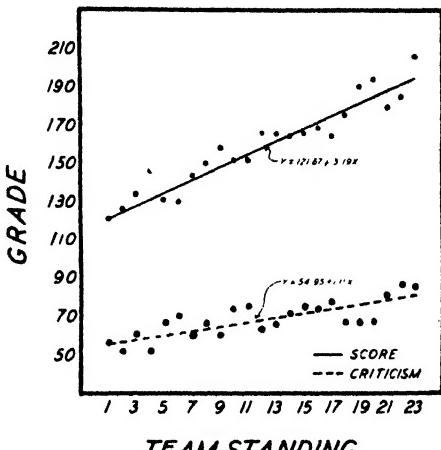


FIG. 5. Lines of regression of team grades on scores and on criticisms in the judging of butter, cheese, milk and ice cream.

#### SUMMARY

Grades on scores and on criticisms of 483 contestant-sample judgments each of butter, cheese, milk and ice cream were studied to ascertain the correlation between scoring ability and criticizing ability.

The mean total grades on butter, cheese, milk, and ice cream were  $14.63 \pm 0.33$ ,  $17.55 \pm 0.52$ ,  $16.97 \pm 0.48$ , and  $26.90 \pm 0.51$  respectively.

Except in cheese judging, the differences in the mean individual grades on criticisms between the groups showing good and showing poor scoring ability were not statistically significant. In cheese judging these differences were highly significant.

The differences in the mean individual grades on scores between the groups showing good and showing poor criticizing ability in judging butter, cheese, milk and ice cream were all statistically significant; those in cheese judging being highly significant.

The differences observed indicate that the judge who can score reliably may not be able to criticize accurately, but that the judge who can criticize the samples fairly accurately may be able to score reliably as well.

Correlation coefficients between all contestant grades on scores and their

grades on criticisms in judging butter, cheese, milk, ice cream, and all products were 0.3064, 0.8214, 0.3193, 0.4456, and 0.2354 respectively; all of which were statistically significant.

Correlation coefficients between team grades on scores and their grades on criticisms in judging butter, cheese, milk, ice cream, and all products were 0.8726, 0.6676, 0.2641, 0.4615, and 0.1981 respectively, the two former being highly significant. The coefficients between team grades on scores and on criticisms of milk and all products were not significant, whereas that of ice cream was significant.

The coefficients of correlation used as a means of predicting the grade on one trait from the grade on another trait gives further indication that the abilities to score and to criticize are not closely similar.

Lines of regression of team grades on scores and on criticisms for butter, cheese, milk and ice cream seem to fit the data very well.

# MINERAL COMPOSITION OF COLOSTRAL MILK<sup>1</sup>

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The unique importance of colostrum in the diet of the new-born calf has made a study of its composition of especial interest to several investigators. Further interest has been stimulated by the usual legal definition of market milk which prevents the sale of milk within 5 days after parturition. It has long been known that colostrum is high in total solids, extremely high in total protein and contains somewhat more total ash, fat, and chlorine and somewhat less lactose than normal milk. It has also been known for some time that the great increase in total protein is due primarily to an increase in lactoglobulin. The work of Crowther and Raistrick (1), Wells and Osborne (2) and Woodman and Hammond (3) is of especial interest in this connection.

Overman and Sammann (4), in 1926 summarized the results on the com-

TABLE 1  
*Summarized results obtained by 25 investigators on cows' colostrum*

	Total solids %	Ash %	Fat %	Total protein %	Lactose %	Specific gravity
<b>First Milking</b>						
No. of Analyses	66	58	73	54	53	59
Maximum	38.40	2.31	9.55	27.35	4.62	1.0830
Minimum	13.72	0.68	0.15	4.80	0.00	1.0318
Average	24.55	1.33	3.89	16.76	2.50	1.0604
<b>Second Milking</b>						
No. of Analyses	45	40	44	42	16	38
Maximum	31.11	1.37	9.00	19.47	4.70	1.0701
Minimum	11.83	0.60	0.50	5.01	2.37	1.0299
Average	18.00	0.97	3.84	9.33	3.52	1.0437
<b>Third Milking</b>						
No. of Analyses	17	16	15	17	12	13
Maximum	27.62	1.25	5.18	17.90	4.44	1.0710
Minimum	12.89	0.67	0.56	4.85	2.74	1.0301
Average	16.75	0.96	3.11	7.06	3.85	1.0376
<b>Fourth Milking</b>						
No. of Analyses	13	9	11	12	9	9
Maximum	26.11	1.24	6.06	6.52	4.80	1.0625
Minimum	12.93	0.77	1.70	4.27	3.62	1.0300
Average	15.21	0.88	3.82	6.16	4.23	1.0372

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<sup>1</sup> The data used in this paper are taken from a thesis presented to the faculty of the graduate school of the University of Illinois by O. F. Garrett in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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position of colostrum as obtained by 25 different investigators. A part of the data presented in table 1 of their article is shown here in table 1.

Eugling (5) in 1875 seems to have been the first to report analytical data showing the ash content of colostrum during its change to normal milk. The more recent work of Engel and Schlag (6), in 1924, and Overman and Sanmann (4), in 1926, is more comprehensive, however, and a summary of their data dealing with ash content is presented in table 2.

TABLE 2  
*Changes in ash content of colostrum*  
*(Data from Engel and Schlag (6) and Overman and Sanmann (4))*

Time after parturition	Engel and Schlag	Overman and Sanmann			
		Holstein	Ayrshire		
			3rd lactation	14th lactation	15th lactation
At once		1.01	1.02	1.02	1.06
3 hours		0.91	0.91	1.02	1.06
6 "		0.91	0.97	1.02	1.02
9 "			0.93		
12 "		0.89	0.86	1.01	0.92
18 "			0.84	1.01	0.87
24 "		0.86	0.82	0.91	0.84
30 "		0.83	0.81	0.91	0.82
36 "		0.84	0.82	0.85	0.79
42 "			0.80	0.85	0.77
48 "		0.83	0.80	0.81	0.77
54 "			0.79	0.81	0.78
60 "			0.79	0.83	0.80
66 "			0.77	0.83	0.80
72 "		0.84	0.77	0.81	0.78
78 "			0.80	0.81	0.77
84 "			0.78	0.80	0.78
90 "			0.79	0.80	0.78
96 "		0.83	0.76		
102 "			0.76		
108 "			0.85		
114 "			0.82		
120 "		0.85	0.78		
126 "			0.79		
132 "			0.80		
138 "			0.76		
144 "			0.77	0.76	0.76
150 "			0.78		
156 "			0.75		
162 "			0.76		
168 "		0.84		0.78	0.76
				0.78	0.78
					0.71

The data of Overman and Sanmann is of especial interest with reference to the data presented in this paper which were also obtained on colostrum from a Holstein and an Ayrshire. The Ayrshire used by Overman and Sanmann, however, was quite an old cow while the one used in these studies was a first-calf heifer.

Engel and Schlag (6) reported that the percentage of  $P_2O_5$ , CaO, MgO and NaCl vary somewhat during the colostral period. Hollen (7) found that the ash of colostrum differed considerably from that of normal milk. His data indicated that calcium and potassium had increased and the sodium decreased by the sixth day while the phosphorus had not reached a normal level.

In the study reported in this paper the milks from a purebred Holstein and a purebred Ayrshire were used. Both animals were first-calf heifers. In collecting the colostrum samples all the milk from two quarters on the same side of the udder was taken. After thoroughly mixing, the milk was analyzed for total solids, total ash, total protein, fat, lactose and chlorides and samples were taken for the mineral element analysis. The latter were dried in large porcelain crucibles and slowly ashed in an electric furnace at a temperature which never exceeded 650° C. Recovery tests of added mineral elements indicated that very little or no loss of elements occurred during the ashing process.

The gross composition of the milks of the two cows are presented in table

TABLE 3  
*Gross composition of colostrum*

Time after calving	Specific gravity	Total solids	Ash	Protein	Fat	Lactose
		per cent	per cent	per cent	per cent	per cent
Holstein						
At parturition	1.0537	27.42	1.37	13.97	8.45	3.63
6 hours	1.0345	27.47	1.07	9.34	13.02	4.04
12 "	1.0316	15.63	0.89	4.77	5.68	4.29
18 "	1.0308	14.56	0.87	4.25	5.26	4.18
24 "	1.0297	13.98	0.87	3.99	4.88	4.24
30 "	1.0304	13.41	0.87	4.09	3.88	4.57
36 "	1.0304	13.54	0.86	3.85	4.08	4.75
44 "	1.0302	13.52	0.85	3.57	4.25	4.85
52 "	1.0297	13.35	0.86	3.66	4.14	4.69
60 "	1.0301	14.22	0.84	3.70	5.02	4.66
68 "	1.0298	14.17	0.84	3.79	5.19	4.35
76 "	1.0314	13.82	0.85	3.86	4.68	4.43
84 "	1.0317	14.70	0.81	3.58	6.79	3.52
11 days	1.0302	12.78	0.75	2.92	4.33	4.78
Ayrshire						
At parturition	1.0594	25.38	1.16	14.70	5.40	4.12
6 hours	1.0457	21.15	1.03	11.36	4.85	3.91
12 "	1.0389	20.32	0.91	7.00	8.46	3.95
18 "	1.0342	14.84	0.83	4.44	4.90	4.67
24 "	1.0343	13.70	0.79	-	4.43	-
32 "	1.0346	11.65	0.87	4.09	2.31	4.38
40 "	1.0345	13.81	0.91	4.17	3.92	4.81
48 "	1.0322	12.22	0.86	4.06	3.14	4.16
56 "	1.0332	12.52	0.82	3.73	3.23	4.74
64 "	1.0321	12.98	0.82	3.83	4.25	4.08
72 "	1.0324	12.87	0.85	3.86	4.00	4.16
10 days	1.0361	13.56	0.75	3.17	3.73	5.91

3. The lactose was obtained by difference (Total solids—(Ash + Protein + Fat)).

At parturition the specific gravity, total solids, total ash, total protein and fat are high. These initial values are followed by a fairly steady decline during the colostral period. It is interesting to note the high fat content in the milks of the two cows respectively at 6 and 12 hours after parturition. The lactose content of these milks is correspondingly low but shows a fairly steady increase as the other milk components decrease.

The percentages of calcium, magnesium, potassium, sodium, phosphorus and chlorine are shown in table 4. Calcium was determined by permanganometric titration of the precipitated oxalate, magnesium by thiosulfate titration after precipitation with 8-hydroxyquinoline and bromination of the precipitate, potassium by permanganometric titration of the precipitated potassium sodium cobaltinitrite, sodium by uranyl zinc acetate precipitation after removal of orthophosphate with solid zinc carbonate, phosphorus by alkali titration of the precipitated ammonium phosphomolybdate and chlorine by a modified Volhard titration.

TABLE 4  
*Mineral composition of colostrum*

Time after calving	Calcium	Magnesium	Potassium	Sodium	Phosphorus	Chlorine
	per cent	per cent	per cent	per cent	per cent	per cent
Holstein						
At parturition	0.256	0.037	0.137	0.074	0.235	0.118
6 hours	0.196	0.027	0.128	0.061	0.178	0.118
12 "	0.154	0.014	0.132	0.051	0.146	0.101
18 "	0.153	0.012	0.139	0.048	0.143	0.098
24 "	0.150	0.013	0.145	0.050	0.137	0.102
30 "	0.151	0.012	0.158	0.050	0.134	0.101
36 "	0.150	0.012	0.154	0.048	0.131	0.103
44 "	0.148	0.013	0.136	0.049	0.127	0.098
52 "	0.154	0.013	0.152	0.054	0.125	0.103
60 "	0.175	0.014	0.170	0.074	0.135	0.105
68 "	0.153	0.012	0.151	0.052	0.125	0.103
76 "	0.176	0.013	0.146	0.065	0.176	0.099
84 "	0.167	0.012	0.174	0.053	0.131	0.099
11 days	0.130	0.011	0.153	0.036	0.113	
Ayrshire						
At parturition	0.206	0.034	0.125	0.079	0.192	0.122
6 hours	0.154	0.012	0.152	0.050	0.123	0.117
12 "	0.142	0.019	0.140	0.072	0.142	0.121
18 "	0.126	0.013	0.153	0.068	0.130	
24 "	0.124	0.013	0.154	0.065	0.129	0.117
32 "	0.133	0.013	0.178	0.052	0.132	0.105
40 "	0.144	0.014	0.181	0.053	0.150	0.091
48 "	0.137	0.014	0.171	0.058	0.137	0.100
56 "	0.126	0.012	0.149	0.054	0.120	0.102
64 "	0.127	0.014	0.161	0.052	0.124	0.094
72 "	0.131	0.015	0.163	0.056	0.125	0.096
10 days	0.120	0.011	0.152	0.047	0.110	0.068

The percentages given in table 4 are for the various elements and not for the oxides of these elements.

Calcium, magnesium, sodium, phosphorus and chlorine are all high at parturition and during the early hours of lactation but a rather rapid decline toward a fairly constant level soon sets in as the milk becomes normal. On the other hand, potassium is rather low at parturition but gradually increases toward a fairly constant level as the milk progresses toward normality.

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# THE USE OF SODIUM METAPHOSPHATE FOR THE PREPARATION OF SOFT-CURD MILK

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## INTRODUCTION

That the physical character of the curd formed upon the ingestion of milk has an influence upon its digestion is generally accepted (1). Ever since the description by Hill (2) of a quantitative method for measuring that property which is presumed to have most direct bearing upon the digestion rate, namely, the curd tension, an increasing amount of work has been done in an effort to arrive at an acceptable method of preparing soft-curd milk for human use.

The most common methods of treating milk to this end are an outgrowth of the frequent necessity for using cow's milk in infant feeding. In attempts to make this food more easily digestible, the addition of various substances such as lime water and gelatin, together with the practice of heating and that of diluting with water, have been quite common. Most of these and similar methods are easily applicable, and there has as a result developed a profusion of "modified" milk formulae for use in the home.

The demand for modified cow's milk in the home has led in turn to a deeper interest in its commercial production. Originally, the only method of obtaining soft-curd milk on a relatively large scale was by sampling the milk from each cow of a herd and separating the milk of the soft-curd producers from that of those cows producing hard-curd milk. The disadvantages of this method are obvious. There followed, however, in rather quick succession other methods by which a dairy might produce soft-curd milk in large quantities. Among these methods are homogenization (3), base-exchange treatment (4) and the use of intense sonic vibration (5). By the first and last of these methods the curd tension is reduced to low values. By the second it is reduced to zero. All three methods have from a general point of view two drawbacks. First, they are not applicable in the home, where, after all, the requirement for soft-curd milk originates; second, when used in the dairy, an appreciable investment in equipment is required, and carefully controlled processing must be assured. A particular disadvantage of the first and last methods is that by their use the "cream-line" is eliminated. From a commercial point of view this may be undesirable. It is the purpose of this paper to report a new method for the treatment of milk

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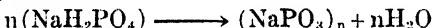
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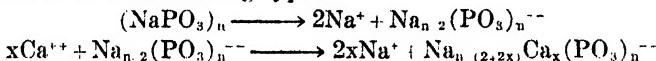
by which milk of zero curd tension can be prepared easily and economically in either large or small quantities.

The method to be described is, in principle, similar to the base-exchange method, in that the net result of the treatment is an apparent decrease in the calcion concentration of the milk. There is this distinction, however; in the base-exchange method there results an actual removal of calcium and phosphorus from the milk. In the method described herein the calcium is not removed, but remains in the milk and is probably bound within a complex ion formed by adding to the milk a small percentage of soluble sodium metaphosphate.

The soluble sodium metaphosphate required for this process is commonly known as Graham's salt, and is a complex, glassy material of approximate composition  $(NaPO_3)_n$ , but of unknown constitution. It results from the fusion and rapid quenching of monosodium orthophosphate in accordance with the following reaction:



This glass is easily soluble in water to the extent of at least 70 per cent by weight. It has the property of reducing the calcion concentration of calcium-containing waters far below the level required for precipitation to occur with the usual precipitating agents. It is this property which has made metaphosphate of considerable value in many technological applications (6, 7, 8), and which undoubtedly makes it possible to prepare with it a soft-curd milk. The mechanism of the reaction between calcium ion and glassy sodium metaphosphate is not thoroughly understood, but it is believed to be of the following type:



#### PART I

(a) *The amount of metaphosphate required to soften milk, both raw and pasteurized.*

Hill's method, as modified by Otting and Quilligan (9) was used for the determination of curd tension. The coagulating medium used by these investigators was an acid solution of pepsin. The calcium chloride used in Hill's coagulant was omitted. This omission of the calcium chloride is entirely reasonable, since, as pointed out by Miller (10), gastric juice contains very little calcium, roughly of the order of .01 per cent, and since further, the calcium chloride in Hill's coagulant retards curd formation and leads to results which are lower than those which are obtained upon coagulating milk with pepsin in hydrochloric acid.

In order to determine the amount of metaphosphate required to soften milk, a given sample of milk was divided into several 100-ml. portions, and to each portion was added metaphosphate<sup>3</sup> in amounts varying from 27 to

70 mg. The curd tension of each portion was measured in accordance with the described technique, and the minimum amount of metaphosphate required to give a milk with no curd was determined. The average amount of metaphosphate required to completely soften 22 different samples of raw and pasteurized milk was 41 mg. per hundred ml. of milk. There was no significant difference between the requirements for the two types of milk. The amounts necessary for each sample were determined to an accuracy of 2 mg. In all of these determinations, the least amount required for complete softening was 30 mg. per hundred ml. of milk, and the greatest amount was 52 mg. per hundred ml. of milk.

It is interesting to note that amounts of metaphosphate less than this yielded milk of zero curd tension as measured, but there was visible a flaky precipitate throughout the mass of milk. The endpoint was therefore taken as that amount of metaphosphate which gave not only a milk of zero curd tension, but also milk with no visible flock of any sort.

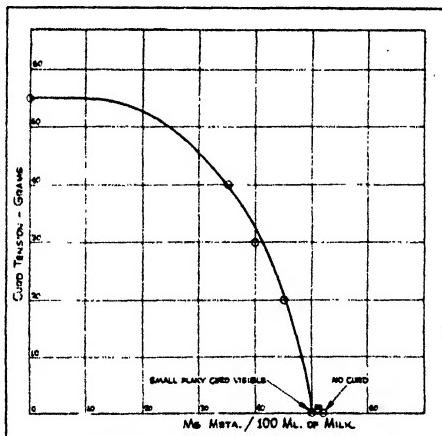


FIG. 1. Variation of curd tension with addition of metaphosphate.

Figure 1 indicates for one specimen of milk the effect upon the curd tension of increasing amounts of metaphosphate. In general, the type of curve for each sample was similar to the figure shown, although the absolute values differed with each milk sample, due probably to differences in the composition of the milk. The results of this type of experiment indicate that the curd tension of milk may within limits be adjusted to any desired value between the original value and zero. For nutritional purposes this is not of importance. In certain commercial operations it may have considerable significance.

<sup>a</sup> The metaphosphate used in all of the work described in this paper was a medicinal grade sold under the trade name "Medi-Calgon," and furnished by Calgon, Inc., Pittsburgh, Pa.

(b) *The amount of metaphosphate required to soften milk with added calcium.*

Following the determination of the minimal amount of metaphosphate required to soften milk, a series of experiments was performed, designed to determine the amount of metaphosphate required to soften milk to which calcium in the form of calcium nitrate or calcium chloride had been added. Data of this type might be valuable as an indication of the possibility of increasing the calcium content of milk, where for therapeutic reasons such increase is considered advisable. In order to determine this, solutions of one or the other of the above-mentioned salts were added to milk so as to

TABLE 1  
*Amount of sodium metaphosphate required to soften completely milk to which calcium was added*

Calcium added mg./100 ml. of milk	Metaphosphate required mg./100 ml. of milk	Calcium added mg./100 ml. of milk	Metaphosphate required mg./100 ml. of milk
0	41	130	600
5	60	140	700
10	79	150	748
15	95	160	800
20	120	170	770
25	140	175	900
30	160	180	1000
40	216	190	950
50	239	200	1025
60	280	210	1100
70	330	220	1150
75	360	225	1200
80	400	230	1200
85	400	240	1200
90	430	250	1205
100	478	280	1350
110	560	300	1400
120	560	350	1550
125	620	450	2200

yield additional calcium varying in amount from 5 mg. to 450 mg. per hundred ml. of milk. The least amount of metaphosphate necessary for giving milk which formed no curd upon treatment with the coagulant was then determined. When the amount of added calcium was between 5 and 10 mg., the metaphosphate requirement was determined to within 2 mg. When the calcium added was between 10 and 20 mg., the metaphosphate additions were varied in steps of 3 mg. When the calcium added was between 20 and 25 mg., the metaphosphate requirement was determined to an accuracy of 5 mg. For every 20 mg. increase in added calcium above this amount, the difference between successive additions of metaphosphate was 5 mg. greater. The results are shown in table 1.

Figure 2 shows the relation between the added calcium and the metaphosphate required for preventing curd formation. Each point is an average of the values found, and resulted from determinations ranging in number from one to fourteen for each value of added calcium. The relation between added calcium and required metaphosphate is, within the limits of accuracy of the experimental procedure, linear. The amount of metaphosphate required to soften milk with added calcium is about 4.8 times as much metaphosphate as calcium, plus an amount of metaphosphate such as will soften the milk itself.

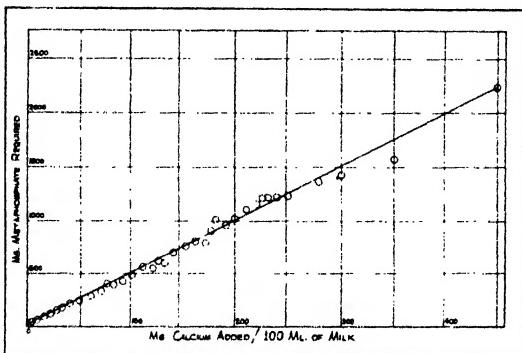


FIG. 2. Metaphosphate required to soften milk with added calcium.

(c) *The effect upon the curd tension of adding calcium to metaphosphate-softened milk.*

Figure 3 indicates the effect upon the curd tension of milk softened with metaphosphate to which additional calcium is added, without increase in the amount of metaphosphate. The drop in the curd tension occurring with the higher values of calcium may be due to the calcium effect mentioned by Miller (10).

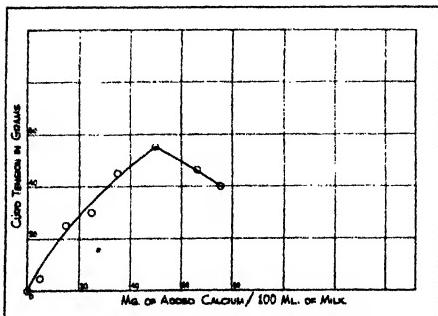


FIG. 3. Curd tension of milk treated with 50 mg. metaphosphate/100 ml. to which calcium is subsequently added.

(d) *The effect of time of contact between coagulant and metaphosphate-softened milk.*

In accordance with the method described herein for determining curd tension, the milk is permitted to remain in contact with the coagulant for a period of 10 minutes before the measurement is made. It was considered advisable to make determinations also with longer times of contact between milk and coagulant, since in the stomach the milk is subject to the action of the gastric secretions for a time considerably longer than 10 minutes. Several 100-ml. portions of milk were treated with 50-mg. portions of metaphosphate and mixed with the pepsin-hydrochloric acid solution. The bottles containing the mixture were kept at 35° C. (95° F.) and curd tension measurements made at intervals. Typical results are shown in table 2.

TABLE 2

*Effect of time of contact with coagulant upon curd tension of metaphosphate-softened milk*

Time of contact between milk and coagulant	Curd tension
10 minutes	0
2 hours	0
4 "	15-20
7 "	50
24 "	70

It may be seen that under the conditions of this experiment milk treated with metaphosphate has no curd tension for 1-2 hours, and for the following two hours may still be considered soft-curd milk, whereas with 7 hours of contact between milk and coagulant the milk has a considerably higher curd tension. Since milk will not normally remain in the stomach for much longer than 4 hours, it might be assumed that in most cases at least, milk treated with metaphosphate will remain relatively soft for that length of time.

(e) *The effect of heat upon metaphosphate-treated milk.*

In order to determine the effect of heat such as might take place in the home during the preparation of a milk formula, or in the dairy during pasteurization, metaphosphate-treated milk was subjected to heating for various temperatures and various times. Samples of milk were treated with 50 and 75 mg. of metaphosphate/100 ml. of milk and divided into two parts. One portion was heated to 60° C. (140°F.) and kept at that temperature for 30 minutes. Another portion was heated to 71° C. (160°F.) for one minute. Both samples were heated in water baths and subsequently cooled by shaking the containers in ice water, and placed in the refrigerator. Immediately after cooling, curd tension tests were run on each sample, and

TABLE 3  
Curd tension of milk treated with metaphosphate and heated

periodically thereafter, additional determinations were made. The results are shown in table 3.

As can be seen from the data, heating has in part nullified the addition of metaphosphate when such addition comprised only 50 mg./100 ml. of milk. Whether these results are due to an alteration in the state of the milk constituents themselves, or whether the heating has caused partial reversion of the metaphosphate to orthophosphate is not known. It seems likely, however, that both factors are involved, since the milk treated with 75 mg. of metaphosphate remained soft for the duration of the experiment, while that treated with the lesser amount was initially soft, then exhibited a moderate curd tension which disappeared upon further standing. The fact that 75 mg./100 ml. of milk was sufficient for complete softening while 50 mg. was not, leads one to believe that reversion has taken place during the heating process. The peculiar behavior of the curd tension of the milk treated with only 50 mg. of metaphosphate/100 ml. of milk might indicate a rearrangement of the milk constituents.

Parenthetically it may be stated that samples of milk treated with this amount of metaphosphate and even higher amounts (up to 250 mg. per hundred ml. of milk) were submitted to about 60 different persons for tasting. No one was able consistently to detect any alteration in the taste or the appearance of the milk.

#### (f) *In vitro digestion experiments*

Following this work it was thought desirable to conduct some experiments in vitro to obtain an approximate comparison between the digestion rate of untreated milk and that of metaphosphate-treated milk. The method used was that described by Doan and Welch (11), and the milk throughout was treated with 100 mg. of metaphosphate per hundred ml. of milk. The average results are indicated in table 4 and figure 4.

TABLE 4

*Digestion rate, in vitro, of metaphosphate-treated and untreated milk*

Time	Metaphosphate-treated milk					Untreated milk				
	pH <sub>2</sub>	pH <sub>3</sub>	pH <sub>4</sub>	pH <sub>5</sub>	pH <sub>6</sub>	pH <sub>2</sub>	pH <sub>3</sub>	pH <sub>4</sub>	pH <sub>5</sub>	pH <sub>6</sub>
0	74%	86%	56%	66%	88%	40%	39%	34%	26%	24%
½ hr.	79	87	63	68	87	52	51	36	30	27
1 "	84	88	77	68	92	55	53	41	32	28
1½ "	95	88	82	72	93	65	54	44	30	28
2 "	95	96	85	72	92	76	58	49	34	27
2½ "	99	95	84	73	92	83	60	48	35	30

Although it is quite apparent that the digestion of metaphosphate-treated milk is consistently greater than that of untreated milk, it is conceded that the results of the experiments indicated are no real measure of

digestion as it occurs in the stomach. Nevertheless, it is reasonable to assume that they are indicative at least of what may transpire upon the ingestion of metaphosphate-softened milk. This is especially so in view of the now well-accepted theory that milk in a fluid state is acted upon more rapidly during the digestive processes than milk permitted to form the characteristic tough, rubbery coagulum.

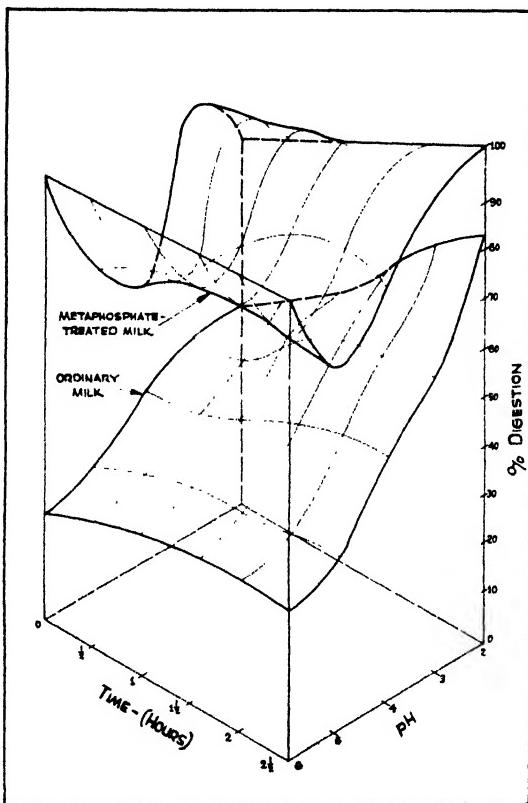


FIG. 4. Comparison of "in vitro" digestion rate of ordinary and metaphosphate-treated milk.

## PART II

### *Experiments, "in vivo," with metaphosphate-softened milk*

Since it is well realized that experiments "in vitro" are only an indication and perhaps a not altogether acceptable one of what might be expected "in vivo," experiments were conducted to determine the effect of the metaphosphate-treated milk on the animal organism. This was considered necessary, since it is obvious that before this method may be used in the preparation of milk for human consumption, it must be shown that milk treated

with metaphosphate is nontoxic and free from deleterious effects upon the body.

It has been shown elsewhere (12) that metaphosphate can induce the symptoms of calcium removal from blood as it does from milk, and that if 40 mg. or more per kilogram of body weight is injected into the blood stream there is a drop in blood pressure. Since this is many times the amount that would be ingested in even the largest consumption of the milk, treated milk would not be dangerous even if the metaphosphate therein would immediately enter the circulation. However, it has been shown, also (12), that when calcium and metaphosphate mixed in vitro, and presumably in the form of a complex, is injected, there is no effect on blood pressure even when it is used in large amounts. This is the form in which the metaphosphate probably exists in treated milk.

An ounce of pure metaphosphate given by mouth to man has produced no further result than a laxative action characteristic of any saline cathartic. In the dog 50 grams were given by stomach tube with only a laxative effect as the result.

It remained to be proved, however, that the long continued ingestion of small amounts does not have a cumulative effect. In order to determine this several different experiments were performed. In the first experiment a litter of 6 rats, 18 days old, was placed on a milk diet supplemented

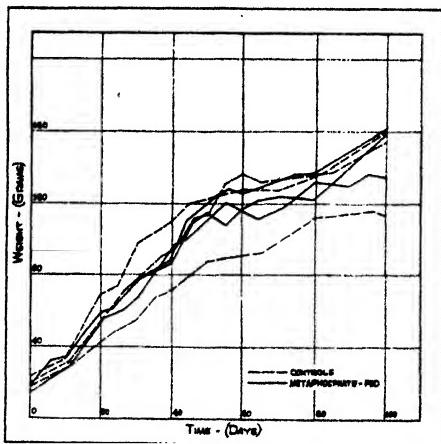


FIG. 5. Growth curves of rats.

daily with a few drops of a solution containing vitamins A, D, E and iron citrate. To the diet of three of these rats, 22 mg. of sodium metaphosphate was added to each 100 ml. of milk. The growth curve and the daily food intake were followed on these rats for three months. The growth curve is shown in figure 5. At 100 days on experiment, a litter of 9 young

was born to a pair fed the metaphosphate-treated milk. These were all weaned, showing that the diet was complete for both reproduction and lactation, one of the most exacting of requirements.

At this time the adult rats were sacrificed and given a thorough macroscopic and microscopic examination. Sections of liver, kidney, heart, spleen, stomach and intestines showed no difference between the rats fed on normal milk and those fed on metaphosphate-treated milk.

In a second experiment 10 adult rats were kept in individual metabolism cages and fed a stock diet containing 0.17 per cent of calcium on the dry basis. To the diet of 5 of these rats sodium metaphosphate was added to the extent of 1:20 of the weight of the food. These rats maintained their weight and ate readily, even though the chemical ingested amounted to 200 mg. per 100 grams of rat weight. After a period of three weeks the amount of metaphosphate was doubled to 1:10 of the diet. The amount of food eaten decreased and the amount of water consumed increased. There was a diarrhea in these rats for 9 days after the increase in the metaphosphate, accompanied by a slight loss in weight. At the end of 9 days the rats returned to a normal condition, with a well formed stool. After 6 weeks on this augmented diet, the rats were killed and autopsied. No difference could be noted between the control and treated rats on macroscopic examination. Microscopic examination showed a questionable fragmentation of tubular epithelium in the kidney.

TABLE 5

*Calcium metabolism of rats on diets with and without metaphosphate*

Days	Urinary calcium (mg. daily)		Fecal calcium (mg. daily)	
	Without metaphosphate	With metaphosphate	Without metaphosphate	With metaphosphate
(1)	0.5852	0.4280	—	—
(2)	0.3138	0.5825	—	—
(3)	0.2641	0.3400	—	—
(4)	0.5905	0.5280	—	—
(5)	0.2112	0.3748	18.5	3.03
(6)	0.2280	0.3465	20.3	3.03
(7)	0.2624	0.2477	26.4	3.04
(8)	1.1165	1.7094	24.3	1.47
(9)	1.2480	Contaminated	25.3	3.60
(10)	1.2846	3.8746	19.3	2.97
(11)	1.7386	5.4797	14.7	2.16
(12)	1.8585	5.4666	16.9	2.34
(13)	1.7145	5.7865	17.4	2.10
Average daily calcium ingested			22.9 mg.	
Average daily urinary calcium	{ normal .....		1.6 mg.	
	{ metaphosphate .....		4.5 mg.	
Average daily fecal calcium	{ normal .....		20.5 mg.	
	{ metaphosphate .....		3.0 mg.	
Average net calcium retention	{ normal .....		0.8 mg.	
	{ metaphosphate .....		15.4 mg.	

Fecal and urinary calcium was followed on this experiment. These results are given in table 5.

The results of these analyses indicate that the addition of the metaphosphate definitely decreased the amount of calcium excreted in the feces and increased the amount excreted in the urine, hence increasing the amount of calcium absorbed from the intestine and the amount retained in the organism. As a check on this, a femur of each of these rats was analyzed for calcium to see whether any storage had occurred. The results of this analysis are shown in table 6.

TABLE 6  
*Femur calcium*

Control rats per cent Ca	Metaphosphate-fed rats per cent Ca
17.8	24.8
18.7	20.5
19.8	24.2
16.1	17.8
17.4	18.9
Average 18.0	21.3

Difference—3.3 per cent.

These results show a slight but definite increase in the amount of calcium in the bones of the metaphosphate-fed rats. As compared to the control rats, the bones of the metaphosphate-fed rats contained about 18 per cent more calcium than the bones of the controls.

The effect of feeding metaphosphate on calcium absorption was next tried on two dogs. The calcium in the food, the feces and urine of these dogs was determined. Each dog was fed eight days on a control diet consisting of one half can of dog food and one half pint of milk, twice a day. Then the dogs were kept on the same diet plus 100 mg. of sodium metaphosphate per kilogram of body weight of the dog. With the addition of the metaphosphate there was an increase in the amount of urine excreted of 40 to 80 ml. per day, even though the fluid intake remained the same. Table 7 gives the results of this feeding test.

On the control diet 44.6 mg. of food calcium was absorbed per day and 20 mg. stored. When on the average 10 grams of sodium metaphosphate was given each dog, an average of 86 mg. of calcium was absorbed and 30 mg. stored. This experiment therefore shows a definite increase in the absorption of calcium during the feeding of the metaphosphate, which increase amounts to about 91 per cent, and an increase in retention of about 50 per cent. The number of experiments was small, however, which left the results open to question.

In order to get more definite information on the absorption and storage of calcium the following experiment was devised. From a strain of rats

TABLE 7  
*Effect of metaphosphate on calcium metabolism in dogs*  
Control

Days	Calcium ingested	Fecal calcium	Urinary calcium
	mg.	mg.	mg.
1	300	257.6	25.52
2	300	240.7	23.90
3	290	220.8	22.70
4	295	270.2	23.20
5	302	261.6	25.90
6	305	254.3	25.10
7	295	259.0	24.30
8	300	263.2	26.80
Average	298.4	253.8	24.68

Average calcium absorbed 44.6 mg. per day.

Average calcium stored 20 mg. per day.

Metaphosphate-fed

Days	Calcium ingested	Fecal calcium	Urinary calcium
	mg.	mg.	mg.
1	305	251.2	36.4
2	300	230.6	37.9
3	295	210.7	45.1
4	302	206.9	70.2
5	298	199.8	64.4
6	300	204.0	63.6
7	300	201.0	65.7
8	300	207.2	65.2
Average	300	213.9	56.0

Average calcium absorbed 86 mg. per day.

Average calcium stored 30 mg. per day.

highly inbred and homogeneous, male rats from the same litter were placed on comparative experiments and fed for the same length of time. The diets fed these litter mates daily were:

- (1) 50 ml. of milk alone.
- (2) 50 " of milk + 75 mg. metaphosphate/100 ml. milk.
- (3) 50 " of milk + 80 mg. iron (in form of ferric ammonium citrate)/100 ml. milk.
- (4) 50 " of milk + iron + metaphosphate in the same amounts as contained in (2) and (3).

Since milk contains approximately 150 mg. of calcium for each 100 ml., it was not necessary to supplement further with calcium. As these rats consumed on the average 40 ml. of milk, they obtained 60 mg. of calcium per day.

At regular intervals a rat from each group was killed. In preliminary tests it was found that the calcium and iron in the food residues of the

TABLE 8  
*Analysis of rats on diets with and without metaphosphate*

	Total avg. calcium in mg. per gram of weight	Probable error	Avg. total iron in mg./ gram of body weight	Probable error
Control	8.34	$\pm 0.198$	0.058	$\pm 0.0006$
Sodium metaphos- phate	9.20	$\pm 0.326$	0.067	$\pm 0.0023$
Sodium metaphos- phate plus iron	8.20	$\pm 0.271$	0.120	$\pm 0.0024$
Iron alone	8.7	$\pm 0.205$	0.097	$\pm 0.0024$

gastro-intestinal tract were sufficient to affect the results, even though the rats had been deprived of food before being killed; therefore, in the later

TABLE 9  
*Summary of average daily weight gain in grams per day*

Day of feeding	Control	Meta- phosphate	Meta- phosphate- iron	Iron
3	0.5	-1.50	-1.0	-1.0
5	1.0	-0.75	-0.5	0.20
7	0	-2.87*	0.67	0.14
9	-0.13	1.25	0.50	1.0
11	-0.2	1.60	0.80	-0.55
13	-0.17	1.67	1.50	1.15
15	-0.38	1.93	1.50	2.9
17	1.88	1.38	2.43	1.5
19	2.61	2.83	1.94	3.1
21	1.35	2.15	1.78	1.71
23	2.36	2.09	2.36	2.6
25	2.25	2.08	1.80	1.70
27	0.7	2.23	1.74	1.80
29	0.18	1.68	1.25	1.41
31	2.03	2.27	1.97	1.90
33	1.88	0.13	1.73	1.82
35	1.65	1.94	2.10	1.50
37	1.72	1.64	1.64	1.80
39	0.18	0.90	1.31	1.08
41	2.06	2.48	2.12	1.90
43	1.49	1.94	1.53	1.44
45	1.18	0.56	0.91	1.11
47	1.09	1.57	1.70	1.70
49	1.38	1.44	1.32	1.55
51	1.90	2.00	1.41	0.96
53	1.75	1.58	1.87	1.85
55	2.16	2.32	2.12	1.94
57	2.14	2.12	3.38	2.14
59	1.52	1.73	1.88	1.49
61	1.81	1.84	2.01	1.46
63	1.3	1.52	1.24	1.30
65	1.54	2.68	2.25	1.46
67	1.08	1.23	-	1.04
Average for series	$1.27 \pm 0.09$	$1.40 \pm 0.12$	$1.54 \pm 0.09$	$1.41 \pm 0.09$

The minus (-) sign indicates a loss in weight.

\* Greater loss than a large series fed on this diet seven days would indicate.

tests the digestive tract below the esophagus was carefully removed. Following this the carcass was weighed and ashed.

On account of the relatively large amount of fat in the rat body, the ashing had to be carried out very carefully to avoid loss. This was done in large quartz dishes over a low flame at first, and later in an electrically heated muffle furnace. When the rat was completely ashed the residue was dissolved in HCl and the solution brought to a known volume so that aliquots could be drawn for analysis of calcium and iron.

In this experiment 132 rats were used, 33 in each series. The first four rats were killed three days after the experiment started, and the last four, after feeding 67 days. The results of this experiment are summarized in table 8.

From these results it is evident that the storage of calcium was not, in these experiments, affected to any marked degree by the addition of the metaphosphate. Iron, on the other hand, was stored to a greater extent

TABLE 10

*Summary of calcium storage in rats in terms of mg./gm. of rat body weight*

Day of feeding	Control	Meta-phosphate	Meta-phosphate-iron	Iron
3				
5	14.53	14.98	14.85	18.95
7	15.8	12.9	11.2	11.82
9	18.5	8.07	9.21	18.93
11	5.6	10.09	9.47	11.7
13	18.2	13.3	8.7	11.93
15	23.1	10.22	9.05	8.9
17	11.00	13.18	12.39	13.02
19	8.16	10.4	7.9	8.52
21	10.5	12.12	9.46	9.06
23	9.36	10.3	8.87	8.23
25	9.34	8.2	9.64	9.76
27	11.05	8.14	10.5	8.95
29	7.74	8.62	10.9	9.33
31	9.05	9.14	9.8	9.68
33	9.80	12.79	8.97	8.5
35	6.55	6.80	7.87	8.53
37	9.25	13.03	8.61	12
39	9.34	9.48	10.9	10.92
41	7.49	7.64	7.92	8.02
43	7.81	10.13	9.24	8.9
45	10.02	16.6	11.85	9.84
47	8.93	9.85	8.23	8.27
49	9.20	10.09	8.40	7.85
51	6.19	6.18	5.97	7.28
53	7.86	6.78	6.8	8.82
55	6.24	6.80	6.16	6.73
57	6.12	6.81	5.98	6.22
59	7.25	7.18	6.79	7.82
61	6.39	6.20	5.52	7.42
63	7.29	7.16	8.10	7.54
65	6.97	6.05	5.42	7.26
67	8.10	8.32	-	8.17

when the metaphosphate was added. It is possible that in the milk-fed rats the calcium absorbed was more than adequate for the demands of growth while the iron on the milk diet was not. The rats, however, gained 0.13 gram more per day on the metaphosphate addition than they did on the control diet, as shown in table 9. A summary of the calcium storage in these rats appears in table 10, that of iron storage in table 11.

TABLE 11

*Summary of iron storage in rats in terms of mg. Fe/gm. body weight*

Day of feeding	Control	Meta-phosphate	Meta-phosphate-iron	Iron
9	0.060	0.101	0.049	0.006
11	0.055	0.064	0.085	0.08
13*	0.098	0.074	0.098	0.116
15	0.060	0.034	0.074	0.068
17	0.059	0.052	0.055	0.051
19	0.093	0.058	0.155	0.073
21	0.072	0.057	0.061	0.108
23	0.049	0.053	0.093	0.063
25	0.035	0.039	0.104	0.075
27	0.044	0.055	0.090	0.075
29	0.058	0.048	0.092	0.076
31	0.051	0.050	0.098	0.097
33	0.053	0.054	0.120	0.085
35**	0.088	0.069	0.093	0.103
37	0.066	0.072	0.111	0.092
39	0.041	0.105	0.090	0.078
41	0.056	0.071	0.095	0.101
43	0.079	0.079	0.118	0.084
45	0.063	0.058	0.101	0.134
47	0.045	0.057	0.0907	0.091
49	0.064	0.089	0.122	0.109
51	0.086	0.114	0.142	0.133
53	0.073	0.087	0.093	0.094
55	0.060	0.094	0.105	0.102
57	0.0423	0.0432	0.0881	0.1069
59	0.0479	0.766	0.0931	0.1278
61	0.0362	0.0366	0.0673	0.075
63	0.0596	0.0606	0.118	0.101
65	0.0538	0.0915	0.1204	0.1288
67	0.0356	0.0506	0.0958	0.1213
	0.0576	0.0523	0.0995	0.0975
	0.0503	0.0621	0.1155	0.1198
	0.0509	0.0550		0.0645

\* Rats fed 13-67 days inclusive had the gastro-intestinal tract removed before ashing.

\*\* All rats of 35-67 day feeding inclusive were run using litter mates for an entire series.

#### SUMMARY

1. Sodium metaphosphate when added to milk in an average ratio of approximately 41 mg. to 100 ml. prevents the formation of a curd when the milk is treated with a pepsin-hydrochloric acid coagulant. For the milk samples used the metaphosphate required to prevent curd formation varied from 30 to 52 mg./100 ml. of milk.

2. When metaphosphate is added to milk before heating, the amount should be increased to 75 mg. per 100 ml. of milk.
3. Additional calcium may be added to milk, and zero curd tension maintained by adding metaphosphate.
4. In vitro experiments indicate that metaphosphate-treated milk is more easily digested than untreated milk.
5. Sodium metaphosphate is nontoxic in the doses used. When added to milk it seems to form a soluble complex with calcium that may be absorbed and utilized by the body.
6. The addition of sodium metaphosphate to a diet decreases the calcium excreted in the feces and increases the calcium excreted in the urine.
7. Sodium metaphosphate increases iron absorption from an iron-containing milk diet.
8. Sodium metaphosphate appears to be a safe, simple and economical means of preparing soft-curd milk either in the home or in the dairy. In contrast to the other methods available, this treatment requires no additional equipment and very little additional labor.

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# THE RELATIONSHIP BETWEEN THE COOKED FLAVOR AND PEROXIDASE REACTIONS IN MILK, SKIMMILK AND CREAM\*

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## INTRODUCTION

Probably the most common method of determining whether or not a reaction is enzymic is to ascertain if the reaction, or the material responsible for the reaction, is adversely affected by heat. In fact, the definition of an enzyme is based partly on the enzyme's heat lability. Consequently, it is the usual practice to determine if a particular reaction will proceed when the material under question has been subjected to high temperatures. If it fails to proceed following the heat treatment, the original reaction is said, often erroneously, to be due to an enzyme. Such a practice may lead to inaccurate conclusions, especially in biological solutions, since the heat treatment will affect the constituents of the biological system itself, and the results of this effect may in itself be responsible for the change in reaction noted.

In another study, the author (3) observed that when milk was heated sufficiently high to cause a cooked flavor, there occurred a simultaneous lowering of the oxidation-reduction potential. Both of these changes were found to be related to the liberation of hydrogen sulfide or to changes which occurred simultaneously with the liberation. During this study, other observations indicated a possible close relationship between the temperature at which the enzyme peroxidase was inactivated and the temperature at which these other changes occurred. Consequently, a further study was conducted to determine if such a relationship existed and, if so, if these changes which were found to occur in heated milk might themselves be responsible for the results secured by the use of the commonly used peroxidase reagents.

Inference has been made that the peroxidase reaction may be influenced by oxidation-reduction changes which may cause condensation of the true meriquinone to that of some other product (1). In addition, -SH groups and hydrogen sulfide are reported to have interfered with peroxidase reactions (2) (9), and it has been further suggested that the peroxidase is perhaps involved in oxidation-reduction systems in which the - SH linkages play a part (6).

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## EXPERIMENTAL

**Procedure:** Mixed-herd milk was heated in four-liter quantities in a round bottom flask suspended in a boiling water bath. An electrically driven glass-rod agitator stirred the milk during the heating period. The rate of heating was regulated so that 15-17 minutes were required to increase the temperature of the milk to approximately 80° C.

Samples were secured in glass sample jars at desired temperatures by means of a glass tube siphon. The samples were immediately cooled by placing the jars in cold water.

When the milk was cooled to approximately room temperature, flavor examinations were made. The milk was examined only for a "cooked" flavor, which has been found to occur abruptly at rather high temperatures and is to be distinguished from the "pasteurized" or "heated" flavor of milk which appears at lower temperatures. Oxidation-reduction potentials of the samples were also determined (3), but only the cooked flavor results are considered in this paper.

Peroxidase determinations were made by use of the Storch test and the Arnold (guaiac) test, according to the recommendations of the International Association of Milk Dealers (7). Strictly, the Storch test as outlined in the I.A.M.D. Manual is modified slightly from the original method in that it specifies p-phenylenediamine hydrochloride instead of p-phenylenediamine. Therefore, in this paper the test is referred to as the modified Storch test. Comparative tests in which those two reagents were used in normal milk indicated no appreciable difference in their reactions. The use of the Storch test seemed particularly desirable inasmuch as p-phenylenediamine appears to be somewhat more sensitive and more constant than other peroxidase reagents (4, 8, 9).

The modified Storch test was conducted using a 0.2 per cent solution of hydrogen peroxide, and a 2 per cent aqueous solution of paraphenylenediamine hydrochloride. Two drops of each of these to 10 ml. of milk, the milk being shaken after each addition, produces a blue color in milk containing active peroxidase.

The Arnold or guaiac test consists of the addition of two drops of hydrogen peroxide and eight drops of a 10 per cent tincture of guaiac to 10 ml. of the milk. A blue color band appears at the junction of the two liquids if peroxidase is present.

**Results:** The close correlation between the cooked flavor, oxidation-reduction potential lowering, and hydrogen sulfide liberation permits any or all of these changes to be considered in connection with peroxidase inactivation. However, for convenience, the cooked flavor findings were used in this comparison.

The results secured in four trials when whole milk was heated for momentary periods at various temperatures are presented in table 1. They

TABLE 1

*The relationship between changes in the peroxidase reactions and the appearance of cooked flavor when milk is heated for momentary periods.\**

Temperature of Heating (° C)	Test	Trial No.			
		1	2	3	4
Raw	Storch	+	+	+	+
	Guaiac	+	+	+	+
	Flavor	0	0	0	0
72	Storch	+	+	+	+
	Guaiac	+	+	+	+
	Flavor	0	0	0	0
74	Storch	+	+	+	+
	Guaiac	+	+	+	+
	Flavor	0	0	0	0
76	Storch	+	?	?	0
	Guaiac	0	0	0	0
	Flavor	++	+	0	+
78	Storch	0	0	0	0
	Guaiac	0	0	0	0
	Flavor	+++	++	+	++
80	Storch	0	0	0	0
	Guaiac	0	0	0	0
	Flavor	+++	++	+	+++

\* Key: Intensity of cooked flavor designated by number of + signs. For the peroxidase tests + represents positive peroxidase reaction, ? represents a questionable reaction, and 0 represents no reaction.

indicate a surprisingly close relationship between the temperatures causing the cooked flavor to appear and those bringing about inactivation of peroxidase as indicated by the Storch or guaiac test. The temperature range of 76-78° C. was sufficient to cause the definite appearance of a cooked flavor and also to bring about a definite change in the color reactions of the reagents.

Milk heated to temperatures lower than 76° C. produced color upon the addition of either the p-phenylenediamine-HCl or guaiac reagents, and in none of these samples was the cooked flavor definitely observed, although one sample heated to 74° C. was scored "questionable" in this regard. When the milk had been heated to 76° C., three of the trials were criticized for a cooked flavor, all four of the samples failed to react to the guaiac test, and three of them gave indications of showing no reaction upon addition of the Storch reagent. In this connection, two of the samples tested with the p-phenylenediamine hydrochloride reagent were graded questionable inasmuch as a color change was produced somewhat slowly, approximately 30 seconds being required after addition of the reagent. In addition, the color change was less distinct than in those samples heated to lower temperatures.

When the temperature of heating reached 78° C. all of the four trials

gave negative peroxidase tests and definite cooked flavors. Higher temperatures produced similar changes.

Several trials were conducted in which the milk was held for 30-minute periods at various temperatures. The results of four of the trials are shown in table 2.

TABLE 2

*The relationship between changes in the peroxidase reaction and the appearance of the cooked flavor when milk is heated for 30-minute periods.\**

Temperature of heating (°C.)	Test	Trial No.			
		1	2	3	4
Raw	Storch	+	+	+	+
	Guaiac	+	+	+	+
	Flavor	0	0	0	0
70	Storch	+	+	+	+
	Guaiac	+	+	+	+
	Flavor	0	?	+	0
72	Storch	0	+	+	0
	Guaiac	0	0	0	0
	Flavor	+	+	++	+
74	Storch	0	0	0	0
	Guaiac	0	0	0	0
	Flavor	++	++	++	+
76	Storch	0	0	0	0
	Guaiac	0	0	0	0
	Flavor	+++	++	+++	++

\* Key: Intensity of cooked flavor designated by number of + signs. For the peroxidase tests, + represents positive peroxidase reaction, ? represents a questionable reaction, and 0 represents no reaction.

Examination of these data shows again, in general, a rather close correlation between the appearance of the cooked flavor and negative peroxidase reactions. The peroxidase tests give positive reactions in raw milk and in the milk heated to 70° C. for 30 minutes. One sample of milk was criticized as being "cooked" when heated to 70° C. and another was graded "questionable" as to flavor. However, when the milk had been heated to 72° C. for 30 minutes, all of the samples possessed a cooked flavor, all of them failed to react to the guaiac reagent and two of them failed to react upon the addition of the p-phenylenediamine hydrochloride. Finally a temperature of 74° C. produced stronger cooked flavors in the milk and the peroxidase tests showed negative results in every case. Again, as was observed in the momentary heating trials, the guaiac test gave negative results at slightly lower temperatures than did the p-phenylenediamine hydrochloride and showed better correlation with the flavor changes. However, the difference between the two reagents was slight.

The results presented in tables 1 and 2 would suggest the possibility that

the reducing system created when milk is heated to 76-78° C. is responsible for the failure of the peroxidase reagents to become oxidized. However, if such is true, then similar relationships between the cooked flavor and negative peroxidase reactions should be indicated by skimmilk and cream. For example, since skimmilk exhibits a cooked flavor at temperatures somewhat above those required for similar changes in milk, and since cream, on the other hand, exhibits this flavor change at lower temperatures (3), the use of these two products may serve as a means of proving or disproving the relationship indicated by the milk studies.

Several trials were conducted in which skimmilk and cream were heated to various temperatures and the peroxidase tests determined upon the samples obtained. Two representative trials for each product are presented in table 3.

TABLE 3

*The relation between changes in the peroxidase reactions and the appearance of the cooked flavor when skimmilk and cream are heated for momentary periods.\**

Temperature of heating (°C.)	Test	Skimmilk		Cream (50%)	
		Trial No. 1	Trial No. 2	Trial No. 1	Trial No. 2
Raw	Storch	+	+	+	+
	Guaiac	-	-	+	+
	Flavor	0	0	0	0
70	Storch	-	-	-	+
	Guaiac	-	-	+	+
	Flavor	-	-	-	?
72	Storch	-	-	+	+
	Guaiac	-	-	+	+
	Flavor	-	-	++	+
74	Storch	+	+	+	+
	Guaiac	+	?	+	+
	Flavor	0	0	++	++
76	Storch	+	+	+	0
	Guaiac	+	+	0	0
	Flavor	0	0	++	++
78	Storch	?	0	0	0
	Guaiac	0	0	0	0
	Flavor	0	0	++	++
80	Storch	0	0	0	0
	Guaiac	0	0	0	0
	Flavor	+	++	+++	++

\* Key: Intensity of cooked flavor designated by number of + signs. For the peroxidase tests, + represents positive peroxidase reaction, ? represents a questionable reaction, 0 represents no reaction. (- represents no sample.)

These results show no apparent relationship between the temperature at which the cooked flavor occurs and those at which the peroxidase reagents

fail to react. In the skimmilk trials, the cooked flavor occurred at 80° C., whereas the peroxidase inactivation occurred within the temperature range of 76-78° C. Likewise, in the case of cream, although the cooked flavor occurred at 70-72° C., no appreciable influence was exerted on the point of peroxidase inactivation, *viz.*, the peroxidase reaction gave negative results in cream heated to 76-78° C. These results show the peroxidase inactivation temperature to be unrelated to the temperature causing the cooked flavor in skimmilk and cream.

In the earlier study (3) observations were made that sodium sulfite and glutathione, when added to milk in the proper concentrations, produced a flavor practically identical with the cooked flavor. Later investigations showed that ammonium sulfite produced a similar flavor. Trials were conducted, therefore, to determine if there was any correlation between changes in the peroxidase tests and this chemically produced "cooked" flavor. The sulfur compounds were added after the milk had been heated and cooled. The two sulfite salts (ammonium and sodium) were added at the rate of 0.5 per cent of 0.05 M solution and the glutathione at the rate of 20 mg. per 100 ml. of milk. Results of a representative trial are presented in table 4.

The chemicals all produced a strong typically "cooked" flavor in the

TABLE 4  
*The influence of sulfur compounds on the peroxidase reaction.\**

Temperature of heating (°C.)	Test	Treatment of sample			
		Control	Sodium Sulfite	Ammonium Sulfite	Glutathione
Raw	Storch	++	+	++	++
	Guaiac	++	++	+++	+++
	Flavor	++	+++	+++	+++
72	Storch	++	+	++	++
	Guaiac	++	++	+++	+++
	Flavor	++	+++	+++	+++
74	Storch	++	+	++	++
	Guaiac	++	++	+++	+++
	Flavor	++	+++	+++	+++
76	Storch	++	+	++	++
	Guaiac	++	0	++	++
	Flavor	++	+++	+++	+++
78	Storch	0	0	0	0
	Guaiac	0	0	0	0
	Flavor	++	+++	+++	+++
80	Storch	0	0	0	0
	Guaiac	0	0	0	0
	Flavor	++	+++	+++	+++

\* Samples heated for momentary periods. Intensity of color produced upon addition of the reagents and the intensity of the cooked flavor indicated by number of (+) signs.

milk. This artificially produced cooked flavor was pronounced even in the raw milk due to the relatively large quantities of the chemicals used. However, no great influence was exerted upon the temperature at which the Storch and guaiac reagents gave negative results. The ammonium sulfite and glutathione gave results not greatly different from those secured from the control sample, both in regard to the temperature to which the milk had been heated as well as the intensity of the color produced in the milk upon the addition of the peroxidase reagents. However, the sodium sulfite often displayed a slight tendency to decrease the intensity of the color produced.

In addition, the sulfite salts and the glutathione had no marked effect upon the oxidation-reduction potential (*Eh*) of the milk, although all of them showed a tendency to bring about a slightly lower potential. The averages of triplicate determinations are as follows:

<i>Sample</i>	<i>Eh</i> (volts)	<i>Difference from normal</i>
Normal milk .....	+ 0.3689	
Milk with sodium sulfite .....	+ 0.3563	- 0.0126
Milk with ammonium sulfite .....	+ 0.3648	- 0.0041
Milk with glutathione .....	+ 0.3607	- 0.0082

These results show a somewhat greater ability of the sodium sulfite to lower the potential than the other compounds used which may account for its greater ability to affect the color produced by the peroxidase reagents. However, in general, these sulfur compounds appear to be inefficient in lowering the *Eh* of milk as well as in affecting the peroxidase reagents.

In addition to studying the effect of the sulfite salts and glutathione on the peroxidase reactions, trials were conducted in which samples of heated milk were subjected to treatment with hydrogen sulfide gas. This was accomplished by bubbling the gas for one minute through milk which had been heated throughout the range of 72–80° C. The milk which was so treated retained a pronounced hydrogen sulfide odor and flavor for several hours following the treatment, indicating that considerable quantities of the gas were retained at least temporarily by the milk. The peroxidase and *Eh* determinations were conducted within a few minutes following the exposure of the milk to the hydrogen sulfide. The purpose of this treatment was to ascertain the effect of this gas in rather large quantities upon the peroxidase reactions, with the view of perhaps showing the possible influence of the hydrogen sulfide which is liberated when milk is heated.

The results of these hydrogen sulfide trials showed that the amount of gas retained by the milk was sufficient to prevent the peroxidase reagents from reacting. This was true not only in the heated milk but also in raw milk which had been subjected to the gas treatment. Further, the hydrogen sulfide markedly lowered the oxidation-reduction potential of the milk. For

example, in one trial the Eh of the untreated raw milk was + 0.3373 volts, whereas it was lowered to - 0.0238 by the addition of the hydrogen sulfide, a decrease of 0.3611 volts. The results indicate that the hydrogen sulfide has marked ability to prevent the oxidation of the peroxidase reagents, and to lower the Eh of the milk. Roemmel (9) has noted that hydrogen sulfide affects the peroxidase reaction.

#### DISCUSSION

When consideration is given to the methods and reagents used to determine the presence or absence in milk of the enzyme, peroxidase, it may readily be seen why questions have been raised as to whether this enzyme is present, or whether these reagents actually indicate some other changes which occur when milk has been heated. The methods of determining the presence of peroxidase are based on the use of reagents which will change color when oxidized by the active oxygen freed from the hydrogen peroxide by the peroxidase. Theoretically, at least, when the temperature treatment is sufficiently high, the reagents will not change color when added to the milk because of the inactivation of the peroxidase and its failure to break down the hydrogen peroxide. Therefore, it is not illogical to suggest a hypothesis that, since the change which these reagents undergo is an oxidation process, the failure of the reaction to proceed in heated milk may be due to the creation of a reducing system, such a system preventing the oxidation of the test reagents. However, the findings secured in this study do not entirely bear out this hypothesis.

The results with the whole milk show excellent correlation between the temperature at which the cooked flavor occurs and those at which the peroxidase reagents fail to react. The correlation holds true both for the momentary and 30-minute heating periods, even though the holding period markedly lowered the temperatures necessary to create the changes under discussion. The lowering of the temperature required to prevent oxidation of the peroxidase reagents by increasing the period of heating is expected since it has been shown that the rate of peroxidase inactivation is dependent upon both temperature and time (5, 10). The cooked flavor temperature is similarly affected (3).

The skimmilk results show no noticeable tendency for the peroxidase inactivation temperature to follow the temperature which brings about a cooked flavor. Higher temperatures were required to cause the cooked flavor to appear, yet the peroxidase inactivation occurred within the normal range of 76-78° C.

The results secured with the cream also show no relationship between the temperatures causing the cooked flavor and those causing a negative reaction with the peroxidase reagents, and offer further contradictory evidence to those secured with the milk. These results must lead one to the conclusion,

at least for the present, that the correlations noted in the case of the milk are mere coincidences, and are not entirely due to other changes involved when milk is heated.

The results with the sulfite salts and glutathione indicate that the addition to milk of certain sulfur compounds which are capable of producing a "cooked" flavor, does not affect the peroxidase reagents and exerts little influence upon the oxidation-reduction potential of the milk. However, hydrogen sulfide, when present in sufficiently large quantities to cause a pronounced odor and flavor in the milk, markedly lowers the Eh of the milk and interferes with the reaction of the peroxidase reagents; a fact which indicates that the hydrogen sulfide formed in milk on heating should not be entirely neglected when consideration is given to tests involving oxidation reagents.

One observation of note in regard to the hydrogen sulfide studies was that the flavor of the milk treated with the gas was not similar to the typical "cooked" flavor, nor to that produced by the use of the sulfite salts and glutathione. This difference may be due entirely to the comparatively large amount of gas which was used. However, it appears more logical to assume that although the cooked flavor of milk may be correlated with sulfide liberation (3), the flavor may not be due to the hydrogen sulfide *per se*, but may be due to unstable sulfur compounds which are formed immediately prior to, or simultaneously with, the hydrogen sulfide liberation.

#### CONCLUSIONS

A close relationship was observed between the temperatures at which the cooked flavor appears in milk and those at which peroxidase is inactivated as detected by p-phenylenediamine hydrochloride and guaiac reagents. A similar but less exact relationship occurred from skimmilk, whereas no correlation between the temperatures was found to exist in the case of cream.

The addition of sufficient quantities of sodium and ammonium sulfite and glutathione to milk to produce an intense "cooked" flavor had no appreciable effect upon the peroxidase reaction nor upon the oxidation-reduction potential of the milk. However, appreciable quantities of hydrogen sulfide greatly affected the oxidation of the peroxidase reagents and markedly lowered the oxidation-reduction potential of the milk.

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## SOME FACTORS AFFECTING CERTAIN MILK PROPERTIES. III. EFFECT OF ROUGHAGES ON ASCORBIC ACID\*

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The possibility that the natural ascorbic acid (vitamin C) of milk may be of greater significance in human nutrition (1) than was once supposed and reports of its importance as a stabilizing factor in the preservation of good flavor in milk have caused an increased interest in the factors which affect the stability of this compound.

Since ascorbic acid is quite readily oxidized, it is relatively unstable in natural solutions and generally becomes increasingly unstable if the natural solutions are processed in any way. If mild conditions of oxidation prevail, the reaction produces dehydro-ascorbic acid which may be returned to the reduced (naturally-occurring) form under proper conditions. On the other hand, if vigorous conditions of reaction exist, the compound undergoes an irreversible oxidation in which the structure of the molecule is completely destroyed.

Reducing or antioxidative reactions seem to have a stabilizing influence on ascorbic acid. Kellie and Zilva (2) found that aqueous extracts of liver, kidney, muscle, spleen, and large and small intestines inhibited the oxidation of ascorbic acid even when copper and iron were added. These natural substances apparently contain antioxidants. Barron, De Meio, and Klemperer (3) reported that the addition of amino acids to solutions of ascorbic acid retarded the catalytic action of copper, and they believed the result was due to the formation of complex salts. It is quite possible that some of the amino acids which contain phenolic groups, such as tyrosine, may act as antioxidants.

There occur in nature certain oxidation-reduction systems which seem to have a profound influence on various natural chemical reactions. Ascorbic acid, itself, forms one of these systems in which the other half of the system is the dehydro-form. Other similar systems which probably occur in milk are cystine-cysteine and glutathione-oxidized disulfide glutathione. Baur and Preis (4) discovered that cystine and cysteine inhibited the oxidation of ascorbic acid in buffer solution of pH 5.3 which contained copper. McFarlane's (5) findings substantiate those above for cystine and cysteine and a compound similar to these two in some respects, sodium diethyldithiocarbamate, is added. Hopkins and Morgan (9) showed that glutathione protects ascorbic acid from catalytic oxidation by copper.

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No report could be found in the literature which directly pertained to the effect of the normal feed of the cow on the stability of ascorbic acid in milk. Whitnah, Riddell and Caulfield (1), however, fed each of 8 cows 0.3 grams of copper daily and found that the stability of vitamin C (ascorbic acid) in the stored milk was significantly decreased. Brown et al (6, 7) found that the feeding of relatively large amounts of ascorbic acid in various forms tended to stabilize the milk against the development of oxidized flavor by copper catalysis without increasing the level of the compound in the milk. They did not indicate whether or not the stability of the ascorbic acid of the milk, while in storage, was affected.

Trout and Gjessing (8) recently found that the rate of disappearance of ascorbic acid, calculated on the third day of titration, was greater in winter-produced than in summer-produced milk. This finding may point to a significant relation between the feed of the cow and stability of the ascorbic acid in her milk since winter and summer feeds vary so greatly.

In experiments previously reported in this Journal, samples of milk taken from cows which were alternately fed beet pulp, corn silage and molasses grass silage as the chief roughage were measured for yellow color and scored for flavor at certain definite intervals of storage. At the same time the ascorbic acid content of each sample was determined by titration with 2,6-dichlorophenolindophenol.

The data presented in table 1 show that the three types of roughage studied have little influence on the amount of ascorbic acid secreted in the milk. While the milk produced on grass silage shows a slightly larger average amount than the milks produced by the other two roughages, the difference does not appear to be highly significant. However, the milk produced

TABLE 1  
*Mean initial content of ascorbic acid of milk produced on different roughages*

Cow No.	All samples	Beet pulp samples	Corn silage samples	Grass silage samples
	milligrams per liter	milligrams per liter	milligrams per liter	milligrams per liter
92 .....	20.31	19.96	19.76	20.37
129 .....	22.29	22.38	21.90	20.74
164 .....	17.54	16.98	18.26	18.39
179 .....	20.53	19.80	20.36	21.04
226 .....	22.36	21.72	22.70	22.42
284 .....	18.49	17.99	17.74	19.50
422 .....	19.29	19.15	19.05	20.89
431 .....	19.11	18.21	20.28	18.49
465 .....	25.72	25.86	23.28	26.90
471 .....	17.47	17.18	17.20	17.62
475 .....	18.10	18.07	16.72	18.55
247 .....	17.47	17.92	16.40	16.59
Mean .....	19.90	19.60	19.47	20.15

Number of samples analyzed in duplicate were as follows: All samples, 215; beet pulp samples, 96; corn silage samples, 36; grass silage samples, 36.

on grass silage was slightly higher in ascorbic acid than that produced on corn silage in 9 out of the 12 cases and higher than that produced on beet pulp in 10 out of the 12 cases.

The percentages of loss of ascorbic acid during 5 days of storage as shown in table 2 indicate that stability of the compound varies considerably with individual cows. There seems to be no definite relationship between the initial concentration of the ascorbic acid and the amount lost during storage.

TABLE 2  
*Loss of ascorbic acid in raw milk during five days' storage*

Cow No.	Initial	Final	Percentage
	concentration	concentration	of loss
	milligrams per liter	milligrams per liter	
92	20.31	13.11	35.30
129	22.29	16.11	27.22
164	17.54	9.89	43.19
179	20.53	11.43	44.14
226	22.36	17.23	22.78
284	18.49	14.04	23.46
422	19.29	13.07	31.80
431	19.11	13.28	29.97
465	25.72	18.67	27.48
471	17.47	13.49	22.55
475	18.10	14.01	22.61
247	17.47	8.51	50.83

\* Each value given in this column is the mean of 18 cases.

The milks used in these studies were pasteurized in 2-liter Pyrex glass flasks. They were protected from diffused sunlight and possible metal contamination at all times. Gentle and continuous rotary stirring was used while the samples were being heated and cooled. The data in table 3 show that when the above method is used pasteurization has only a slight influence on the loss of ascorbic acid in milk during storage.

TABLE 3  
*Loss of ascorbic acid in 215 samples each of raw and pasteurized milk during 5 days of storage*

Mean initial concentration and probable error	Mean loss and probable error	
	Raw milk	Pasteurized milk
milligrams per liter	per cent	per cent
19.90 $\pm$ 0.14	31.82 $\pm$ 0.60	33.47 $\pm$ 0.67

A study of the data presented in table 4 reveals that the type of roughage in the diet of the cow influences the stability of the ascorbic acid in storage. The ascorbic acid in the milk, either raw or pasteurized, produced on grass silage showed the least loss during 5 days of storage while that of milk pro-

duced on beet pulp showed the greatest loss. Similar data, not presented here, bear out this finding for a 3-day-storage period. This result seems to be in line with the findings of Trout and Gjessing (8) mentioned earlier in this paper.

TABLE 4

*Loss of ascorbic acid during 5 days of storage as influenced by type of roughage*

Type of roughage	Mean loss and probable error	
	Raw milk	Pasteurized milk
	per cent	per cent
Beet pulp	32.72 ± 0.93	35.90 ± 0.98
Corn silage	30.72 ± 1.51	29.82 ± 1.62
Grass silage	27.56 ± 1.34	27.17 ± 1.16

Number of samples analyzed: Beet pulp, 96; corn silage, 36; grass silage, 36.

The percentages of loss for 5 days of storage of ascorbic acid in milks produced on the different roughages were segregated into class intervals in order to observe the distribution of losses. These data are presented in tables 5 and 6.

TABLE 5

*Distribution of losses of ascorbic acid in raw milk as influenced by type of roughage*

Class interval	Percentage of cases in each class		
	Beet pulp	Corn silage	Grass silage
<i>per cent</i>			
0 -10.00	2.08	2.78	.....
10.01-20.00	11.46	22.23	25.00
20.01-30.00	34.37	27.78	33.33
30.01-40.00	27.08	27.78	30.56
40.01-50.00	16.66	13.89	5.56
50.01-60.00	4.17	2.78	5.56
60.01-70.00	2.08	2.78	.....
70.01-80.00	1.04	.....	.....
80.01-90.00	1.04	.....	.....

Number of samples analyzed: Beet pulp, 96; corn silage, 36; grass silage, 36.

TABLE 6

*Distribution of losses of ascorbic acid in pasteurized milk as influenced by type of roughage*

Class interval	Percentage of cases in each class		
	Beet pulp	Corn silage	Grass silage
<i>per cent</i>			
0 -10.00	.....	8.34	5.56
10.01-20.00	11.46	19.45	19.44
20.01-30.00	30.21	33.34	33.34
30.01-40.00	21.88	16.67	80.55
40.01-50.00	18.75	11.11	11.11
50.01-60.00	11.46	8.33	.....
60.01-70.00	4.17	2.78	.....
70.01-80.00	2.08	.....	.....

Number of samples analyzed: Beet pulp, 96; corn silage, 36; grass silage, 36.

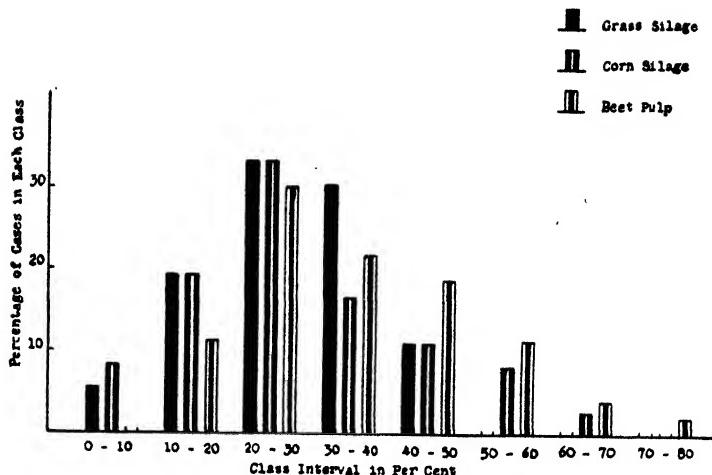


Figure 1 - Distribution of Losses of Ascorbic Acid in  
Pasteurized Milk as Influenced by Type of Roughage

#### SUMMARY

The data presented in this paper indicate that:

So far as the three types of roughage are concerned, none has a significant influence on the amount of ascorbic acid secreted into the milk.

Factors associated with the individual cow have a marked influence on the stability of ascorbic acid while the milk is in storage.

If milk is pasteurized so as to protect it from violent agitation, sunlight and metal contamination, the loss of ascorbic acid during storage is not significantly greater than that of the corresponding raw milk.

The feeding of properly ensiled molasses grass silage has a greater stabilizing effect on ascorbic acid of milks in storage than does corn silage or beet pulp. This conclusion is in line with the earlier findings that grass silage is superior to the other two roughages in producing milk with yellow color and good flavor.

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## A MODIFICATION OF THE RITTER TEST FOR COPPER IN BUTTER<sup>1</sup>

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It is generally known that dairy products contaminated with copper will develop specific off-flavors on aging or in storage. The more common flavor defects which have been observed are tallowy, fishy, metallic, oily, cappy or oxidized and rancid flavors.

Copper, in the amounts found in butter or other dairy products, is not harmful from a nutritional standpoint, but will induce the production of off-flavors because of its catalytic and oxidative reactions. The copper in dairy products not only speeds up the production of these flavor defects, but also helps to destroy some vitamins; and it has been reported to inhibit the normal growth of the acid-producing bacteria, thus accelerating putrefaction.

With these facts in mind an investigation was started to study the extent of copper in various dairy products. This article is a study of the copper content in Indiana butter.

The importance of copper contamination in milk has been studied by a number of investigators. However, most of the investigational work reported by various workers of the effect of copper on the keeping qualities of butter has been done by the addition of definite amounts of copper. Very few results have been reported on the actual amount of copper contamination that takes place during the manufacture of butter.

Rogers, *et. al.*, (1) and Humziker (2) showed that very small amounts of copper were capable of producing changes in butter held in storage in that metallic and other related flavor defects developed.

Davies (3) found that factory-made butter contained more copper than hand-made butter. His analysis of butter from New Zealand and Australia ranged from a trace to 3.7 p.p.m. and one sample of whey butter had 9.3 p.p.m. copper. His results show that butter having a high copper content was prone to have undesirable flavors. Williams (4) studying New Zealand butter found the normal content of superfine butter to contain 0.2 to 0.25 p.p.m. of copper and that second grade butter was about the same; however, whey butter had a much higher copper content.

Ritter (5) developed and modified the peroxidase-reaction test for the detection of copper in butter or butter-oil by heating the emulsifying agent (milk not contaminated with copper) to 90° C. for one to three minutes to kill or inactivate the enzyme. To 10 ml. milk he added one to two grams of

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butter. The reagents were added to this mixture and incubated at 45° C. He found that the greater the copper contamination of the butter, the less time it took to develop a blue color.

#### EXPERIMENTAL

During the past two and one-half years, we have been experimenting with several tests for the determination of copper in dairy products. The various tests investigated were found to be for milk and cream or liquid dairy products and consisted of long procedures. However, one was found, namely, the Ritter Test (5) which showed promise and was relatively simple. This test was tried on butter, but with variable results. From this investigation, a test has been devised that will determine copper contamination in butter qualitatively.

The following test which is a modification of the Ritter Test has been used this past year on Indiana butter in order to determine the extent of copper contamination. Our modification of this test consists in using a 10 gram sample of butter as it comes from the print, tub or churn, thereby omitting the addition of milk (copper free). The test has been standardized for length of time for the development of the color in the water-curd portion of the sample. The following technique was used:

About 10 grams of unmelted butter were placed in a test tube and one-half ml. of copper-free distilled water added. This mixture was heated at 90° C. for five minutes to kill or inactivate the peroxidase.

Solutions: 1), 0.2 gram para-amino-dimethyl-anilin sulfate was added to five ml. of copper-free water (glass distilled) and heated to 90° C. for five minutes in the presence of one gram of bone black and then filtered. (Made fresh when needed.) 2), One per cent of alpha naphthol in alcohol. 3), Hydrogen peroxide one per cent in glass distilled water.

To the melted butter one drop of each of the above solutions was added, shaking after the addition of the first two. Then one drop of the hydrogen peroxide solution was added and shaken again. The separation of the curd and time the color began to appear was then observed. In the very positive copper samples, the purple color appeared almost at once, positive in about five minutes and traces in 10 to 15 minutes. We have found that if the color did not develop in 15 minutes, the sample of butter was negative in copper.

In order to justify the test, it was compared with a quantitative determination of copper in butter (6) (7). Table 1 shows the range of copper and the average in parts per million according to the classifications of the reading of the modified Ritter method. The negative, slight and positive classifications were used to designate the extent of copper contamination of the butter. There was some overlapping of the actual copper content as read by the qualitative test. However, on the average, the copper content increased as shown by these readings.

TABLE 1

*Comparison of the modified Ritter test and quantitative determination of copper*

No. of tests	Modified Ritter	Quantitative p.p.m.	
		Range	Average
52 .....	Negative	0.1 -0.58	0.229
65 .....	Slight	0.19-0.54	0.291
41 .....	Positive	0.32-2.33	0.583

In the course of our investigation it was found that the pH of the butter serum seemed to have some influence on the test. To check this influence, various amounts of N/10 H<sub>2</sub>SO<sub>4</sub>, and N/10 NaOH were added to 50 gram samples of the same butter. After melting, about 10 grams of this butter were placed in test tubes for the modified Ritter Test. The pH of the serum was determined electrometrically using a quinhydrone electrode. The results are shown in Table 2.

TABLE 2

*Influence of the pH on the modified Ritter test*

Sample	pH	Modified Ritter	Remarks
Check + 6 ml. N/10 H <sub>2</sub> SO <sub>4</sub>	2.90	++	Fat darkened
" + 4 ml. "	3.62	++	Fat darkened
" + 2 ml. "	4.56	+	Fat darkened
" + 0.6 ml. "	5.10	++	Fat darkened
Check (50 gms.)	5.32	+	
" + 0.6 ml. N/10 NaOH	5.76	+	
" + 1 ml. "	6.00	+	
" + 2 ml. "	6.50	Sl. to +	
" + 3 ml. "	6.90	-	
" + 4 ml. "	7.60	-	
" + 6 ml. "	8.30		Slight suspension of curd in the fat Suspension of the curd in the fat

+ positive; ++ very positive; - negative.

It will be noted in Table 2 that from a pH of 2.90 to 5.10 the test showed a very positive copper contamination and the fat was also darkened, from a pH of 5.32 to 6.50, a positive test, and at 6.90 and above, a negative test. This darkening of the fat was also noticed in commercial butter with high copper even though the pH was below 6.5. Other tests were run on samples showing negative by the modified Ritter Test and if only a small amount of copper was present the addition of a small amount of acid did not materially change the negative reading. Turgeon, Stebnitz and Sommer (8) found the degree of acidity to have some influence on the Ritter Test in milk. They stated, "At high acidities the test failed to detect the presence of even fairly large amounts of copper." Our results show with the modified test for butter that increased acidity intensified the color and this intensified color appeared in a shorter time when fairly large amounts of

copper were present, but had no effect on butter with low amounts of copper.

Difficulty was encountered with some butter samples in that the curd remained suspended in the fat after heating. It was found on examining the pH of the butter serum that the serum had a high pH. These samples in nearly all cases gave a negative reading according to the modified Ritter Test even though the butter contained a high copper content.

After our observation that acid increased the color of the modified Ritter Test in the presence of fairly high amounts of copper, the addition of acid was tried on butter samples showing suspension of curd in the fat. Table 3 shows the results of a few of the typical tests made by the use of acid on the butter samples showing suspension of the curd in the fat. These samples in all cases showed a high pH in the butter serum. By the addition of acid, a true indication of the copper contamination of the butter was revealed, while without the addition of the acid, the modified test gave erroneous results.

TABLE 3

*Effect of the addition of acid to the butter sample containing various amounts of copper on the modified Ritter test to decrease the pH*

Modified Ritter	Modified Ritter + N/10 H <sub>2</sub> SO <sub>4</sub>	Copper p.p.m.	Original pH of butter serum
-	SI.	0.34	6.90
-	++	1.12	7.35
-	+	0.42	7.50
-	+	0.54	7.05
-	+	0.56	6.85
-	++	0.90	6.90
-	-	0.31	7.30
-	-	0.33	7.50

- negative; slight; + positive; ++ very positive.

#### RESULTS

The examination of 591 samples of Indiana butter with the modified Ritter test for copper contamination during the year 1938 is shown in Table 4. These samples were from 51 creameries from all parts of the State of Indiana and were sent in monthly by the creameries. Table 4 gives the number of samples by months showing negative, slight and positive copper contamination with the modified Ritter Test, also the percentage of samples in each classification for the year. The data show April, May and December to have the largest number of samples with positive copper. There seems to be no month in which there was an extensive contamination nor one in which there was very little contamination. This could be expected since the samples were sent in by the operator and also that the first run cream from equipment with copper exposed would have more copper contamination than succeeding churings of cream. The data in Table 4

reveal that over a period of a year, 21.3 per cent of the samples had positive contamination of copper.

TABLE 4

*Copper contamination of Indiana butter by months as shown by the modified Ritter test*

Month	No. of samples		
	Negative	Slight	Positive
January	17	23	12
February	23	21	10
March	25	17	9
April	26	13	14
May	16	16	16
June	14	22	10
July	11	21	11
August	22	20	7
September	18	21	9
October	26	18	6
November	27	18	6
December	9	21	16
Per cent	39.6	39.1	21.3

In this study of copper contamination of butter, keeping quality tests were also made on each sample. The butter was scored as it was received and then rescored after an incubation temperature of 70° F. for seven days. The results in Table 5 are from two creameries and were obtained from monthly samples taken consecutively from each creamery during the year 1938. The equipment used by Creamery No. 1 was made of tinned copper. However, most of the tin had been worn off the forewarmer and coil, holding vats and cooling equipment. The equipment of Creamery No. 2 was well-tinned with just a few scratches of copper showing. It will be noted that the butter from Creamery No. 1 was of lower score when received than was that of Creamery No. 2. This tendency for relative high copper contaminated butter to be of lower score was observed in the results from samples of other creameries. The pH tended to be somewhat lower in the butter of Creamery No. 1 and the copper content ranged from 0.5 to 4.0 p.p.m., while the copper content of the butter from Creamery No. 2 ranged from 0.14 to 0.52 p.p.m.

The results in Table 5 show that copper contamination of butter lowers the keeping quality. The fact that butter with the higher copper content was of lower quality when received may be partly due to this contamination and also due to the fact that there was little attention paid to the grading, sanitation and processing of the product. The results from other butter with somewhat lower copper contamination showed that the butter did not deteriorate so rapidly as the butter from Creamery No. 1. Therefore, from the results it would appear that copper contamination of butter in combination with careless grading and processing can only result in a lower

TABLE 5

*Copper contamination of butter from two creameries and its effect on the keeping quality*  
Creamery No. 1

Copper (Ritter)	In score	Remarks	Out score	Remarks
+	89.0	Old cream, sl. bitter	88.0	Stale
++	89.5	Sl. old cream	88.0	Sl. cheesy, musty
+++	88.0	Sl. onion	86.0	Sl. onion, metallic, sl. cheesy
+++	88.5	Old cream, sl. bitter	87.5	Very stale
+++	88.0	Old cream, sl. neut.	86.0	Very stale, sl. cheesy
++	88.0	Sl. yeasty, old cream, sl. bitter	87.0	Sl. rancid, stale
+++	88.0	Old stale cream, sl. cheesy	86.0	Cheesy, stale
++	88.0	Cheesy, yeasty	87.0	Rancid
+	88.0	Old cream, bitter, sl. yeasty	87.0	Cheesy
++	88.0	Stale cream, sl. cheesy	85.0	Fishy
++	88.0	Old, stale cream	86.0	Cheesy, musty
+++	88.5	Sl. cheesy	86.0	Cheesy, sl. musty, rancid

## Creamery No. 2

Modified Ritter				
Sl.	90.5		90.0	
Sl.	90.5		90.0	
V. Sl.	91.0		90.5	
+	88.0	Onion	88.0	Onion
Sl.	91.5	High flavor	90.0	
+	91.0	Not fine	89.5	Sl. old
Sl.	91.0		89.0	Old
-	92.0		91.5	Sl. storage
-	92.5		91.5	
-	90.5		90.0	
-	90.0		87.5	Cheesy
-	90.5	Very high flavor	90.0	Sl. old

grade of butter and with poor keeping qualities. In other words, the authors do not believe that the copper contamination of butter is the chief cause of the poorer keeping quality but tends to speed up the deterioration due to the catalytic and oxidative properties of the copper.

## DISCUSSION

The Ritter Test was modified to make a short, rapid, qualitative test for copper in butter. Three different classifications were made in the reading of the test to determine the extent of the copper contamination: Negative reading, in which the butter contained on the average 0.221 p.p.m. of copper and showed no bluish purple color in the curd at the end of 15 minutes; slight, which had a slight bluish purple color in the curd at the end of 15 minutes and in which the butter contained on the average 0.291 p.p.m. of copper; and positive, which had a deep bluish purple color in 15 minutes or less and contained on the average 0.583 p.p.m. of copper.

The results in Table 2 show that the pH influenced the test, the more

acid in the sample the deeper the color down to about a pH of 5.0; then there was no color change, but the color was developed more quickly in the lower pH range. It was found that in most butters with a pH of above 7 in the serum, the curd would be suspended in the fat after heating and would give a negative test even though the copper content was high. By the addition of a very small quantity of N/10 H<sub>2</sub>SO<sub>4</sub> to change the pH as well as to break up the suspension, a true reading of the test could be made which compared very favorably with the actual copper content of the butter for the reading observed.

The data obtained with the use of this modified test on a year's study of Indiana butter reveal that 39.6 per cent of the samples were classified as negative copper contamination, 39.1 per cent as slight and 21.3 per cent positive copper. This would indicate that many of the plants have equipment that should be retinned.

Table 5 shows the effect of copper contamination in the butter from one creamery as compared with the butter from another creamery with practically no contamination. Our results show that the butter with high copper was of poorer quality than butter with low amounts of copper contamination. The highly contaminated samples had inferior keeping qualities and the butter was prone to develop musty, stale and rancid flavors. However, the lower quality of the butter with heavy copper contamination suggests the lack of attention to methods of sanitation and manufacture. It is probable that factors other than copper contamination played an important part in the poor keeping quality of this butter and that copper contamination was not the chief cause, but may have been a contributing factor in the rapid deterioration of this butter.

#### SUMMARY

1. A modified Ritter Test for copper in butter has been described.
2. Three classes of readings for this copper test have been proposed to designate the extent of the copper contamination in the butter.
3. The test is influenced by a high pH of the butter serum, but may be corrected by the use of a small quantity of N/10 H<sub>2</sub>SO<sub>4</sub>.
4. The copper contamination of Indiana butter was found to be 39.1 per cent negative, 39.6 per cent slight and 21.3 per cent positive according to the modified test.
5. The comparison of butter of high copper contamination from one creamery and that of another creamery with very little contamination was made.
6. This modified test although qualitative is fairly accurate in determining the extent of copper contamination in butter and is also a rapid, inexpensive test.

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# THE INFLUENCE OF STABLE TEMPERATURE ON THE PRODUCTION AND FEED REQUIREMENTS OF DAIRY COWS

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## INTRODUCTION

Animal husbandmen employed by agricultural colleges and experiment stations, as well as other leaders working for improved animal husbandry, have been prone to assume that farm animals should be comfortably housed and that if they are kept in cold quarters, they will produce less and would have to use more or less of their ration to maintain body temperature. Many farmers apparently never have been and are not now overly careful about keeping their stock in warm quarters.

*Beef Cattle.* Waters (1), writing in 1907, stated that "It has long been assumed that animals exposed to cold are required to use a considerable part of their ration to maintain the normal temperature of the body, and that a considerable part of the food used for fuel may be saved by providing a warm shelter for the animals." That year he sent out a questionnaire to beef cattle feeders and found that about 18 per cent of them used a warm barn, nearly 60 per cent an open shed, and about 23 per cent an open lot for feeding beef cattle. Many of the open sheds used were reported to afford little shelter.

The summary of a three-year trial by Waters (1) showed that cattle housed in barns required 10.77 pounds of dry matter, cattle in open sheds required 10.25 pounds, and cattle in an open lot with no shelter other than windbreak and with cornstalks for bedding required only 10.22 pounds of dry matter for a pound of gain. Thus states Waters, "The cattle confined in barn at night and during stormy weather ate less, made smaller gains and less gain per pound of dry matter consumed than cattle that had access to an open shed or less economical gains than did those which were in an open lot without shelter and a pile of cornstalks to lie on." Other trials (2, 3, 4, 5, 6, 7, 8, 9) at various stations confirmed these results.

Armsby (10), after reviewing the experimental evidence available at that time, 1908, noted that a summary of the experiments covering full fattening periods shows fully as good results for the exposed as for the barn-fed animals. "It seems clear at least that the value of shelter for fattening cattle has been exaggerated."

*Dairy Cattle.* It has also been assumed that of all farm animals, the dairy cows are the least able to withstand exposure and therefore must be kept in warm, comfortable quarters. Plumb (11) in 1893, Henry and Morrison (12) and Eckles (13) in 1923 expressed somewhat the same sentiment, although Eckles stated that dairy cows, "when well fed are not so sensitive to low temperature as is sometimes assumed."

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An explanation published by Armsby (14) in 1917 has been quite generally overlooked or ignored. Using experiments by Kellner, he showed that of the energy evolved as heat by a fattening steer on full feed, about 62 per cent of it was used to maintain body heat and 38 per cent was surplus. Armsby then cited work by Jordon (15) to show that the heat produced by one dairy cow was greater by 85 per cent and for another was 54 per cent greater than the amount needed for maintenance and that, "so far as mere maintenance of body temperature goes, no reason appears why a cow might not be subjected to comparatively low temperatures without causing any increased katabolism for the sake of heat production solely."

Buckley (16) at Maryland compared open stables to closed stables and found that, "the effects of extremely low temperatures are practically negative in reducing the flow of milk."

Davis (17) compared an open shed with a barn for milk cows in Pennsylvania and concluded that drops in atmospheric temperature decreased the milk yield for both groups, and that the cows in the open shed consumed slightly more roughage than the cows kept in the barn. Woodward, *et al.* (18), also compared the open shed with the closed barn for milk cows and report that "the cows consumed somewhat more feed and produced slightly more milk when kept in the open shed than when kept in the closed barn."

During the time this project was in progress, Kelley and Rupel (20) studied the relation of stable environment to milk production and found that under Wisconsin conditions the optimum stable temperature for dairy cows appeared to be about 50° F. and that cows running loose in a pen barn withstand low temperatures better than stanchioned cows. Regan and Richardson (21) housed cows in an air conditioned room and demonstrated that heavy milking dairy cows withstand cold temperatures better than warm temperatures.

#### EXPERIMENTAL

The preliminary work on the project reported in this paper was done to demonstrate the folly of turning milk cows out in the yard in the morning during cold weather and leaving them out all day. The production and persistency of production of this group of five cows was compared with that of a similar group kept in the barn and turned out for exercise for a few hours on nice days. All of the cows were kept in the barn during the night. At the end of two months, November-December, we were surprised to find that the out-of-doors cows maintained their production on a par with those kept in the barn.

The mean temperature for November was 22° F., for December 9° F., with the minimum going below zero several times.

The barn cows gained 184 pounds in live weight and the yard cows gained 207 pounds during the two-month period.

Table 1 gives the production of five cows in each group whose production

TABLE I  
*Production of cows housed in the barn vs. cows kept in yard during day*

TYP BARN

*Month before	Trial period		*Month after		*Month before		Trial period		*Month after	
	Oct.	Nov.	Jan.		Oct.	Nov.	Dec.		Jan.	
			Nov.	Dec.			Nov.	Dec.		
<b>Butterfat</b>										
lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Mabel .....	40.6	30.6	33.3	24.8	1127.3	987.5	934.6	934.6	798.9	798.9
Melba .....	16.8	16.6	17.4	16.5	480.3	448.4	457.5	457.5	421.9	421.9
Sophia .....	44.9	35.3	32.7	22.5	956.6	705.2	594.0	594.0	449.1	449.1
Bunburst .....	18.3	19.5	20.2	19.7	398.3	375.5	367.1	367.1	344.8	344.8
Louise .....	15.1	12.5	12.5	11.3	260.6	215.4	196.0	196.0	166.6	166.6
Per month .....	135.7	114.5	116.1	94.8	3223.1	2732.0	2549.2	2549.2	2181.3	2181.3
Per day .....	4.4	3.8	3.7	3.1	104.0	91.1	82.3	82.3	70.3	70.3
Per cent of the October production .....	86.4	84.9	84.9	70.5	87.6	79.0	67.6	67.6		
<b>Milk</b>										
<b>Out of doors during day</b>										
(In Barn) *										
Alice .....	39.9	41.9	43.1	38.0	1597.8	1446.3	1368.3	1368.3	1267.9	1267.9
Cynthia .....	23.8	24.7	24.1	18.8	700.6	707.2	643.6	643.6	469.8	469.8
31.5 .....	31.5	30.2	29.8	27.5	1049.9	973.0	961.3	961.3	888.7	888.7
22.2 .....	22.2	21.9	20.4	20.6	411.3	354.7	342.9	342.9	332.1	332.1
Raleigh .....	18.8	12.4	10.5	6.9	417.0	317.0	245.0	245.0	165.0	165.0
Per month .....	136.2	131.1	127.9	111.8	4176.6	3798.2	3561.1	3561.1	3123.5	3123.5
Per day .....	4.4	4.4	4.1	3.6	134.7	126.6	114.9	114.9	100.8	100.8
Per cent of the October production .....	100.0	93.2	81.8	81.8	94.0	94.0	85.3	85.3	74.8	74.8

\* All cows housed in dairy barn.

records were available for the month before and the month after the trial period.

To check these results other groups of cows were turned out, night and day, during February and March and again during November and December with access to hay in a rack and to an open shed. They were milked and fed grain and silage in the barn. The production of these groups was compared with that of similar groups housed in the dairy barn.

The mean temperatures for February and March 1930 were 23.0° F. and 27.0° F., respectively and for November and December were 30.4° F. and 19.8° F. Table 2 and table 3 show the results of the two comparisons.

TABLE 2  
*Milk production by groups (converted to 4% milk)*

IN BARN

	1930		1930	
	Feb.	Mar.	Nov.	Dec.
No. cows included .....	3	3	4	4
Lbs. of 4% milk produced .....	2484.83	2537.73	3464.47	3201.36
Daily production of 4% milk .....	88.74	81.86	115.48	103.27
Lbs. drop in daily production .....		6.88		12.21
% drop in daily production .....		7.75		10.57

IN SHED

	3	3	4	4
No. cows included .....	3	3	4	4
Lbs. of 4% milk produced .....	2751.61	2833.66	3483.64	3144.79
Daily production of 4% milk .....	98.27	91.41	116.12	101.44
Lbs. drop in daily production .....		6.86		14.68
% drop in daily production .....		6.98		12.64

TABLE 3  
*Average daily milk production by groups (converted to 4% milk)*

IN BARN

	No. cows	Daily production of 4% milk	
		First month	Second month
Feb.-Mar. 1930 .....	3	88.74	81.86
Nov.-Dec. 1930 .....	4	115.48	103.27
Total .....	7	204.22	185.13
Lbs. drop in daily production .....	7		19.09
% drop in daily production .....	7		9.35
Lbs. daily production per cow .....	7	29.17	26.45

IN SHED

Feb.-Mar. 1930 .....	3	98.27	91.41
Nov.-Dec. 1930 .....	4	116.12	101.44
Total .....	7	214.39	192.85
Lbs. drop in daily production .....	7		21.54
% drop in daily production .....	7		10.05
Lbs. daily production per cow .....	7	30.63	27.55

The production of the cows is calculated to 4 per cent milk using the formula 0.4 M plus 15 F.

As indicated in table 4, the cows in the shed gained more weight than the cows in the barn.

TABLE 4  
*Weight summary*

No. cows	Barn cows	Shed cows
	Lbs. gain or loss	Lbs. gain or loss
Feb.-Mar. 1930 .....	3	- 92
Nov.-Dec. 1930 .....	4	+ 340
Total .....	7	+ 248
Av. gain per cow .....		+ 35

#### NUTRIENTS REQUIRED

These data seemed to indicate that the cows housed in cold quarters produced practically as well as those kept in a standard dairy barn.

Contrary to the statement by Armsby (14), the assumption had been that the cows in the cold quarters required more feed than those in the dairy barn. The next phase of the project was to compare the amount of nutrients required by a group of cows housed in a cold shed where the door was left open except during stormy weather and where the temperature was below freezing with that used by another group of cows or the same cows housed in the dairy barn. The cows in the shed were fed grain and silage and milked in the dairy barn.

The comparisons for the first two trials were between cows kept in the barn vs. cows kept in the shed. The period used was for five months beginning November 1. The results are summarized in table 5.

TABLE 5  
*Production of separate groups of cows*

	1932-33		1934-35	
	Barn cows	Shed cows	Barn cows	Shed cows
Lbs. 4% milk produced .....	19,664	18,393	18,521	20,297
Mean humidity .....			84.30	75.86
Mean temperature, degrees F. ....	45.30	31.80	51.16	24.87
Lbs. gain in weight .....	292	426	459	556

The producing ability, stage of lactation, size and breed of the cows were always considered in making up the groups. During the next two seasons the method was changed somewhat and one group was kept in the dairy barn while the other group was in the shed and then the next month the two groups were reversed. This plan served to overcome any of the inevitable differences that existed in the two groups.

TABLE 6

*Same cows in barn and shed—alternate months*

	1935-36		1937-38	
	Barn cows	Shed cows	Barn cows	Shed cows
Lbs. 4% milk produced .....	16,358	16,305	27,910	27,887
Mean humidity .....	83.50	73.40	82.92	67.23
Mean temperature, degrees F. ....	52.20	25.95	53.90	34.96
Lbs. gain or loss in weight .....	+ 363	+ 440	- 134	+ 1199

Combining the results obtained in the four comparisons, we have the summary in table 7.

TABLE 7

*Summary of all trials*

Summary of 4 trials (1932-33, 1934-35, 1935-36, 1937-38)		
	Barn cows	Shed cows
Lbs. 4% milk produced .....	82,453	82,882
Mean humidity .....	83.57	72.16
Mean temperature, degrees F. ....	50.64	29.39
Gain in weight, lbs. ....	+ 980	+ 2621

TABLE 8

*Feed consumed*

	Barn cows			Shed cows		
	Hay	Silage	Grain	Hay	Silage	Grain
		lbs.	lbs.		lbs.	lbs.
1932-33 .....	9,570.5	28,904	5,485.0	8,775.0	26,347	4,680.0
1934-35 .....	10,961.0	32,483	4,563.0	10,628.0	31,700	4,362.0
1935-36 .....	10,415.0	29,302	3,597.0	10,392.0	29,300	3,352.0
1937-38 .....	14,682.0	40,897	6,441.0	14,445.0	40,957	6,322.0
Total .....	45,628.5	131,586	20,086.0	44,240.0	128,304	18,716.0

TABLE 9

*Digestible nutrients consumed*

	Barn cows		Shed cows	
	Digestible crude protein	Total digestible nutrients	Digestible crude protein	Total digestible nutrients
	lbs.	lbs.	lbs.	lbs.
1932-33 .....	1,840.9	13,998.11	1,654.0	12,555.98
1934-35 .....	1,942.0	14,872.80	1,880.0	14,409.60
1935-36 .....	1,060.6	12,930.10	1,636.0	12,738.98
1937-38 .....	2,599.9	19,287.91	2,570.3	19,044.24
Total .....	8,043.4	54,038.92	7,740.3	58,748.80

During these trials the cows were fed corn silage and alfalfa hay in proportion to their body weight and were fed grain according to their production using Woodward's rule (19) as a guide. The grain ration consisted of oats, barley, corn, wheat bran, bone meal and salt.

TABLE 10  
*Digestible nutrients used for production of milk*  
SEPARATE GROUPS OF COWS

	1932-33		1934-35	
	Barn cows	Shed cows	Barn cows	Shed cows
Lbs. protein for 100 lbs. 4% milk	9.36	8.99	10.49	9.26
Lbs. total digestible nutrients for 100 lbs. 4% milk	71.19	68.26	80.30	70.99
Same cows in barn and shed—alternate months				
	1935-36		1937-38	
Lbs. protein for 100 lbs. 4% milk	10.15	10.03	9.32	9.22
Lbs. total digestible nutrients for 100 lbs. 4% milk	79.04	78.12	68.93	68.29
Summary of all trials (1932-33, 1934-35, 1935-36, 1937-38)				
Lbs. protein for 100 lbs. 4% milk			9.83	9.37
Lbs. total digestible nutrients for 100 lbs. 4% milk			74.86	71.41

#### HEALTH OF COWS

No cases of pneumonia developed during any of the trials. The shed cows did not suffer from frosted teats as much as was anticipated and when teats were frosted, it was in most cases due to wading thru snow in coming and going from the shed to the barn at milking time. Slightly more mastitis was reported for the groups in the shed and there was no difference in the occurrence of "off feed" cases.

It would therefore seem that under North Dakota conditions the following conclusions would be warranted.

#### SUMMARY AND CONCLUSIONS

1. The idea that dairy cows receiving an adequate ration need to be kept in a warm barn to be comfortable seems to be an assumption rather than a fact.
2. Data presented herein show that provided dairy cows receive an adequate ration, have shelter from the wind, snow or rain and have a dry place to bed down that: (a) they can withstand exposure to cold temperature; (b) that they will produce practically the same in a cold stable as they will in a

stable where the temperature is about 50° F.; (e) that milk cows on full feed, when housed in a cold stable produce sufficient surplus heat over usual maintenance requirements to maintain body temperatures without using nutrients for that purpose; (d) that cows housed in a cold shed require if anything somewhat less protein and total digestible nutrients for milk and butterfat production than other cows or the same cows when kept in a standard dairy barn; (e) that the cows in the cold shed tend to gain somewhat more body weight than the cows in the dairy barn; (f) that the comfort and convenience of the caretaker and the protection of watering systems rather than the need of the cows justify the use of stables that are common today.

3. It should be noted that the cows kept in the cold shed were always loose while those in the dairy barn were always kept in stanchions.

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## LIVE WEIGHT AND MILK-ENERGY YIELD IN CZECHOSLOVAK COWS

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A recent paper<sup>1</sup> by Kleinberg presents some 14,000 records of cows in Czechoslovakia, dealing with age, live weight, milk yield and fat yield. Kleinberg's analysis of these records includes an application of the equation,<sup>2</sup>  $M = A + BW$  and the equation,  $F = \Delta + BW$ . One of his three major breed groups and several of his minor age groups give a negative value for  $\Delta$ , which indicates that in these particular groups the larger cows produce more milk or more fat *per unit live weight* than do the smaller cows. This situation is of so much interest that it seems desirable to express the results in terms of a power equation, relating milk-energy yield to live weight. Accordingly, in the present paper Kleinberg's published data are fitted with the equation,

$$\mathbf{FCM} \neq \mathbf{bW}^e \quad \text{and} \quad \mathbf{FCM} \neq \mathbf{bW}^d \quad (1)$$

In fitting equation (1) the constants,  $b$  and  $c$  are derived from a least-squares solution of  $\log FCM = \log a + c \log W$ , taking the  $W$ 's as the midpoints of the live-weight classes, and the  $FCM$ 's as the average  $FCM$ 's of the live-weight classes. The classes are treated as of equal weight, that is, differences in  $n$  are disregarded. The result is not a least-squares fit of equation (1) but affords an acceptable approximation to it.

MILK-ENERGY YIELD AS A POWER FUNCTION OF LIVE WEIGHT

Table 1 presents the constants of equation (1) for various groups of records according to breed and age of cow. The values of the exponent,  $c$ , range<sup>3</sup> from .10 to 2.96. Excluding groups of less than 100 records, the range is from .50 to 1.62.

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<sup>1</sup> Kleinberg, Antonin. Studie o vlivu velikosti (váhy) a věku dojnice na producií mléka (mit deutscher Zusammenfassung). Sborník výzkumných ústavů zemědělských ČSR. Sv. 162. Praha, nákladem ministerstva zemědělství, 1937.

\* Symbols are used in the present paper as follows:

$W$  = live weight of cow, kg.

M = milk yield for fiscal year, kg.

**F = fat yield for fiscal year kg.**

**FCM = .4M + 15F** = milk-energy yield for fiscal year, kg. 4% milk. (1 kg.  
4% milk = 750 calories.)

n = number of cows or records

<sup>3</sup> When working maintenance is expressed as a power function of live weight a similar range in value of the exponent (viz., .15 to 2.30) is found, with the further similarity that the best all-round value appears to be unity.

TABLE 1  
*Milk-energy yield as a power function of live weight*

Breed	Range in age yrs.	Range in weight kgs.	n	FCM = bW <sup>c</sup>	
				b	c
All breeds .....	2-15*	310-790	13,807	4.045	1.021
Berner .....	2-15*	370-790	653	.5067	1.368
Pinzgauer .....	2-15*	310-710	4,785	13.51	.802
Simmentaler .....	2-15*	310-790	8,367	12.09	.850
Berner .....	2-4	390-690	85	113.4	.499
Pinzgauer .....	2-4	310-630	478	8.449	.871
Simmentaler .....	2-4	310-770	1,074	6.013	.956
Berner .....	5-6	390-790	160	.6610	1.332
Pinzgauer .....	5-6	310-710	1,062	60.55	.528
Simmentaler .....	5-6	310-790	2,361	9.849	.884
Berner .....	7-8	390-790	111	.1034	1.618
Pinzgauer .....	7-8	310-710	1,105	18.26	.754
Simmentaler .....	7-8	310-790	1,967	15.28	.816
Berner .....	9-10	430-790	59	.1090	1.596
Pinzgauer .....	9-10	310-710	754	6.567	.920
Simmentaler .....	9-10	310-790	935	7.716	.919
Berner .....	11-12	490-710	33	.000408	2.460
Pinzgauer .....	11-12	310-790	252	11.50	.818
Simmentaler .....	11-12	370-750	329	134.3	.504
Berner .....	13-15	490-690	18	.0000138	2.961
Pinzgauer .....	13-15	330-630	83	147.5	.405
Simmentaler .....	13-15	410-750	82	1343	.104

\* The starred groups include some cows of unknown age.

The three breeds have exponents in equation (1) of 1.37, .85, and .80 respectively. By combining the three breeds (without regard to differences in n) the 13,807 records give the equation  $FCM = 4.045 W^{1.021}$ . The data for the three breeds and the combined results are shown graphically in Figures 1, 2, 3 and 4.

#### DISCUSSION

The results for the cows represented in table 1 and figures 1-4 effectively dispose of the notion that "active mass" or "metabolic body size" or "physiologic weight" is proportional to the  $\frac{1}{2}$  power of live weight in the sense that the  $\frac{1}{2}$  power of live weight represents the *limit* of potential capacity of cows to do sustained work in lactation. On the other hand the results lend support to the notion that, as between cows of different live weights, potential lactation capacity (present or capable of development) is proportional to live weight.\*

\* The validity of this concept is not disturbed by the fact that small species as compared with large species of mammals may produce milk energy at a faster rate per unit

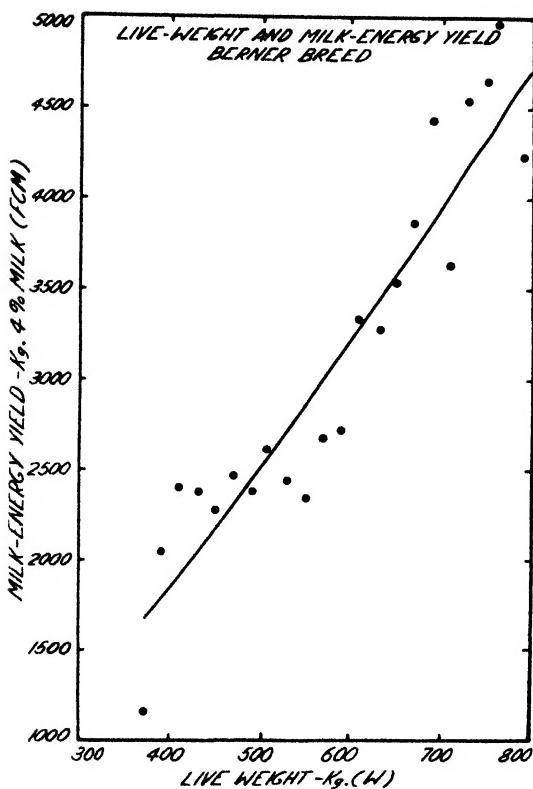


FIG. 1. Relation of milk-energy yield to live weight in the Berner breed, 653 records.  
Equation of the smooth curve,  $FCM = .5067 W^{1.368}$ .

A noteworthy feature of Kleinberg's data is the great range in live weight—from 300 to 800 kilograms. This range is equivalent to that from a small Jersey (660 pounds) to a large Holstein (1760 pounds). It is amazing to find such a range of size existing within a single breed, and, furthermore, within a narrow age range within a single breed (*e.g.* the group of 935 Simmental cows, 9–10 years of age, table 1).

From the standpoint of number of records and range of live weight involved, Kleinberg's data rank high as bearing on the problem of the relation between live weight of cow and milk-energy yield.

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*live weight at the flush of lactation.* In general, small species live faster and shorter lives than large species. A fair comparison between species with respect to milk-energy yield per unit live weight must introduce length of life. The lifetime capacity of organisms to work (transform energy in the various life processes) may be in general proportional to mature live weight, while the mature capacity for rate of work (as between species) may be proportional to the  $3/4$  power of live weight.

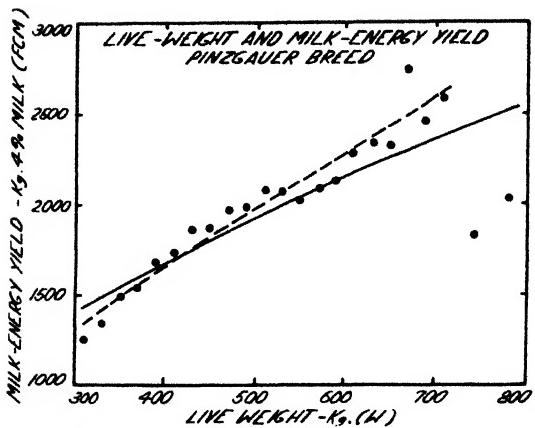


FIG. 2. Relation of milk-energy yield to live weight in the Pinzgauer breed, 4,787 records.

Equation of solid line,  $FCM = 40.79 W^{.620}$ . Omission of the last two observations ( $n = 1$  in each) gives the broken line,  $FCM = 13.51 W^{.802}$ .

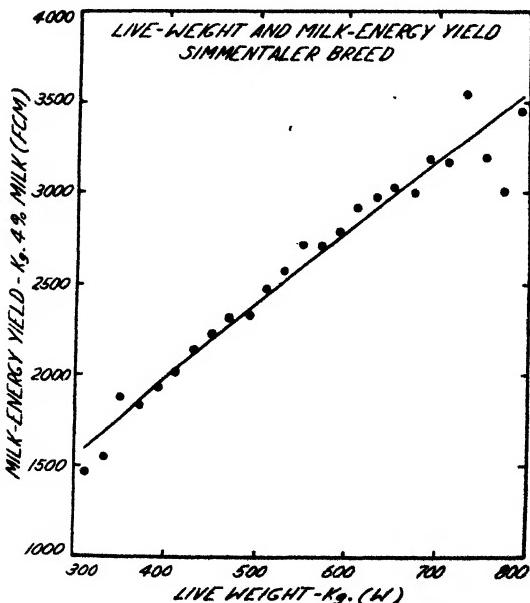


FIG. 3. Relation of milk-energy yield to live weight in the Simmentaler breed, 8,367 records.

Equation of the smooth curve,  $FCM = 12.09 W^{.880}$ .

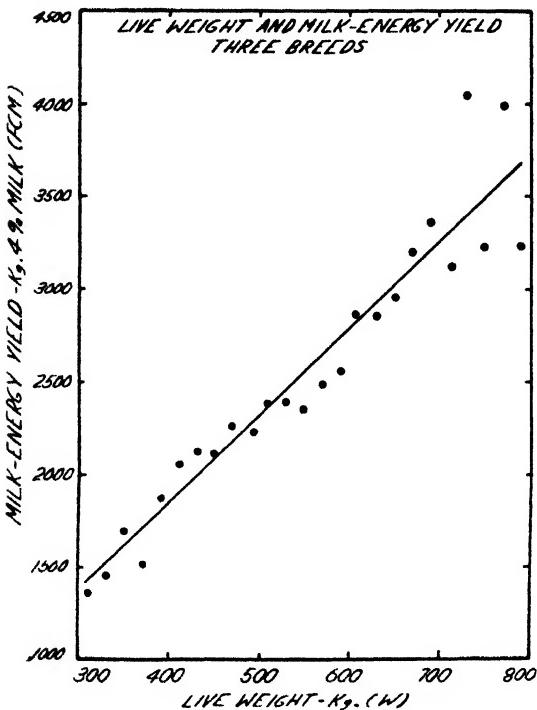


FIG. 4. Relation of milk-energy yield to live weight in three breeds, 13,807 records.

Equation of the smooth curve,  $FCM = 4.045 W^{1.021}$ . The data are obtained from those of Figures 1, 2 and 3, disregarding differences in  $n$ , and including the last two observations of Figure 2.

#### SUMMARY AND CONCLUSIONS

Kleinberg's data comprising 13,807 records of cows of three breeds are fitted with the equation,  $FCM = bW^c$ , where  $FCM$  is milk-energy yield, kg. 4% milk per year, and  $W$  is live weight of cow, kg. The records as a whole give the equation  $FCM = 4.045 W^{1.021}$ . For the three breeds the exponent,  $c$ , is respectively .802, .850 and 1.368. For various age groups within breeds the exponent ranges from .10 to 2.96. If groups of less than 100 records are excluded the range of exponents found is from .50 to 1.62.

The number of records and range of live weight (300 to 800 kgs.) make the data particularly valuable as bearing on the relation of milk-energy yield to live weight in the cow. The results support the equity of using milk-energy yield per unit live weight as a measure of dairy development among cows.



## REPORT OF THE STUDENTS' NATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

*San Francisco, Calif., October 23, 1939*

The Iowa State College team was victorious in a big way at the Products Judging Contest held at the Dairy Industries Exposition in San Francisco, in which 14 teams participated. The Iowa team won first place in judging butter, milk, and ice cream and, therefore, first in "all products." The trophies were 4 silver cups and a Dairy Industrial Research Fellowship. Individual members of the team won first, second, and third places in "all products," first and third in butter, first and third in ice cream, and first in milk. Awards to individuals are gold, silver, and bronze medals for first, second, and third places, respectively.

The University of Wisconsin team won the cheese judging event, beating Iowa, however, by only  $\frac{1}{2}$  point.

Dairy Industrial Research Fellowships for winning second and third places in "all products" were awarded to the University of Wisconsin and Mississippi State College.

Following are additional contest data:

Teams from fourteen (14) State Agricultural Colleges participated in this, the tenth annual contest sponsored by the Dairy Industries Supply Association, Inc., and the American Dairy Science Association.

Following is a list of those who won high standings in the contest:

### ALL PRODUCTS

#### *Individuals*

1—Donald E. Sherman, Iowa State College . . . . .	80.05
2—Orel M. Russell, Iowa State College . . . . .	88.85
3—Raymond J. Nelson, Iowa State College . . . . .	97.75
4—T. J. Goodwin, Mississippi State College . . . . .	98.01
5—Herb. Hollender, University of Wisconsin . . . . .	102.60
6—Marshal C. Winton, University of Tennessee . . . . .	103.91
7—T. A. Young, Mississippi State College . . . . .	106.76
8—Paul H. Cober, Pennsylvania State College . . . . .	106.80
9—Richard Neutzling, Ohio State University . . . . .	106.83
10—James M. Nunnally, University of Tennessee . . . . .	110.95

#### *Teams*

1—Iowa State College . . . . .	5
2—University of Wisconsin . . . . .	12
3—Mississippi State College . . . . .	13
4—Pennsylvania State College . . . . .	20
5—University of Tennessee . . . . .	22
6—Ohio State University . . . . .	29
7—South Dakota State College . . . . .	33

7—University of California .....	33
9—University of Minnesota .....	34
10—Texas Technological College .....	35
11—University of Nebraska .....	36
12—Kansas State College .....	43
13—New Mexico A. & M. College .....	51
14—Texas A. & M. College .....	54

**BUTTER***Individuals*

1—Raymond J. Nelson, Iowa State College .....	7.5
2—John W. Walch, Pennsylvania State College .....	8.5
3—Ocrel Russell, Iowa State College .....	9.5
4—Donald E. Sherman, Iowa State College .....	9.5
5—T. J. Goodwin, Mississippi State College .....	10.0
6—Loren Zook, University of Nebraska .....	10.5
7—Alvin Rippen, University of Nebraska .....	11.0
8—Clayton Pflueger, South Dakota State College .....	11.5
8—Paul H. Cober, Pennsylvania State College .....	11.5
10—Orville H. Herrick, University of California .....	12.5
10—Marshal C. Winton, University of Tennessee .....	12.5

*Teams*

1—Iowa State College .....	26.5
2—University of Nebraska .....	37.5
3—Mississippi State College .....	44.0
4—University of Wisconsin .....	45.0
5—South Dakota State College .....	47.5
6—Pennsylvania State College' .....	50.5
6—Ohio State University .....	50.5
6—University of California .....	50.5
9—University of Tennessee .....	51.0
10—University of Minnesota .....	59.5
11—Kansas State College .....	67.5
12—Texas Technological College .....	71.5
13—New Mexico A. & M. College .....	79.0
14—Texas A. & M. College .....	104.0

**CHEESE***Individuals*

1—Marshal C. Winton, University of Tennessee .....	25.25
2—Herb. Hollender, University of Wisconsin .....	25.25
3—Paul H. Cober, Pennsylvania State College .....	25.25
4—Loren Zook, University of Nebraska .....	25.50
5—Donald E. Sherman, Iowa State College .....	27.25
6—John R. Raup, Pennsylvania State College .....	27.50
7—Ocrel M. Russell, Iowa State College .....	29.00
8—Owen Pilgrim, University of Wisconsin .....	29.25
9—Orville Nellen, University of Minnesota .....	29.75
9—Willie Bell, Texas Technological College .....	29.75

*Teams*

1—University of Wisconsin .....	86.75
2—Iowa State College .....	87.00
3—University of Tennessee .....	88.75
4—Pennsylvania State College .....	91.25
5—Mississippi State College .....	92.25
5—Texas Technological College .....	92.25
7—University of Minnesota .....	93.50
8—Kansas State College .....	97.25
9—Ohio State University .....	97.75
10—University of Nebraska .....	101.25
11—University of California .....	111.00
12—South Dakota State College .....	112.75
13—New Mexico A. & M. College .....	134.00
14—Texas A. & M. College .....	144.25

## ICE CREAM

*Individuals*

1—Orel M. Russell, Iowa State College .....	32.0
2—Donald E. Sherman, Iowa State College .....	33.5
3—T. J. Goodwin, Mississippi State College .....	37.5
4—James Jezeski, University of Minnesota .....	38.0
4—James M. Nunnally, University of Tennessee .....	38.0
6—Raymond J. Nelson, Iowa State College .....	38.5
7—Orville H. Herrick, University of California .....	39.5
7—Paul H. Cober, Pennsylvania State College .....	39.5
9—John W. Walch, Pennsylvania State College .....	39.66
10—Richard Neutzling, Ohio State University .....	39.83

*Teams*

1—Iowa State College .....	104.00
2—Mississippi State College .....	125.00
3—University of Wisconsin .....	127.00
4—Pennsylvania State College .....	127.66
5—University of Tennessee .....	129.50
6—South Dakota State College .....	130.81
7—University of California .....	133.00
8—University of Minnesota .....	136.82
9—Texas Technological College .....	137.16
10—Kansas State College .....	138.16
11—Ohio State University .....	139.65
12—University of Nebraska .....	147.50
13—Texas A. & M. College .....	152.00
14—New Mexico A. & M. College .....	173.50

## MILK

*Individuals*

1—Donald Sherman, Iowa State College .....	9.8
2—Thomas Harman, Ohio State University .....	15.15

3—T. A. Young, Mississippi State College .....	16.51
4—John D. Bowers, Ohio State University .....	16.93
5—Herb. Hollender, University of Wisconsin .....	17.85
6—Oerel M. Russell, Iowa State College .....	18.35
7—T. J. Goodwin, Mississippi State College .....	19.01
8—Raymond J. Nelson, Iowa State College .....	21.00
9—Marshal C. Winton, University of Tennessee .....	22.66
10—John R. Raup, Pennsylvania State College .....	22.85

*Teams*

1—Iowa State College .....	49.15
2—Ohio State University .....	55.08
3—Mississippi State College .....	60.92
4—University of Wisconsin .....	74.93
5—University of Tennessee .....	77.86
6—Pennsylvania State College .....	78.45
7—University of California .....	80.76
8—Texas Technological College .....	81.72
9—University of Minnesota .....	91.82
10—South Dakota State College .....	98.11
11—New Mexico A. & M. College .....	100.05
12—University of Nebraska .....	104.16
13—Texas A. & M. College .....	110.76
14—Kansas State College .....	116.11

## American Dairy Science Association Announcements

PURDUE UNIVERSITY INVITES YOU TO ATTEND THE THIRTY-FIFTH ANNUAL  
MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

*To the Officers and Members of the  
American Dairy Science Association:*

It is a pleasure to know that the Thirty-fifth Annual Meeting of the American Dairy Science Association will be held at Purdue University, June 24-28, 1940. We hope that you will take this opportunity to become acquainted with the members of our staff and to visit our different departments while you are on our campus.

You may also be interested in visiting some of our fine Indiana farms and factories, and at least one of the State Parks for which Indiana is noted.

We are looking forward to your coming to Purdue and extend to you a most cordial welcome. We will make every effort to assist you in having a successful meeting and to see that your visit with us is a pleasant one.

Very sincerely yours,

(Signed) EDWARD C. ELLIOTT, Pres.

Purdue University,

W. Lafayette, Indiana

THIRTY-FIFTH ANNUAL MEETING, PURDUE UNIVERSITY,  
W. LAFAYETTE, INDIANA, JUNE 24-28, 1940

*Monday—June 24*

- 1:30 P.M.—General Registration and Room Assignment.  
2:00-4:00 P.M.—Dairy Products Judging Conference for Coaches and Instructors.  
8:00 P.M.—Board of Directors Meeting.

*Tuesday—June 25*

- 10:00-12:00 Noon—Opening Session.  
1:30-3:30 P.M.—Sectional Meetings.  
3:30 P.M.—Committee Meetings.  
8:00 P.M.—Social Hour.

*Wednesday—June 26*

- 8:30-9:30 A.M.—Committee Meetings. Inspection of Extension Exhibits.  
9:30-12:00 Noon—Sectional Meetings.  
1:30-3:30 P.M.—“ “ “  
3:30 P.M.—Business Meetings for Sections.  
8:00 P.M.—Entertainment.

*Thursday—June 27*

- 8:30-9:30 A.M.—Committee Meetings.  
9:30-12:00 Noon—Sectional Meetings.  
1:30-3:30 P.M.— “ “ “  
3:30 P.M.—General Session and General Business Meeting.  
6:30 P.M.—Annual Association Banquet.

*Friday—June 28*

- 9:00 P.M.—Board of Directors Meeting.

**CALL FOR TITLES AND ABSTRACTS OF PAPERS**

Members of the Association are invited to submit titles and abstracts of papers dealing with original investigations. Those interested in presenting papers should comply with the rules regarding the abstracts and other information listed below.

**RULES REGARDING ABSTRACTS AND PAPERS**

Any member of the Association may submit a title for the program. Members are limited to two papers of which they are authors or co-authors unless the extra papers fit into the program being planned. No title will be accepted unless it is accompanied by an abstract. At least one of the authors of a paper must be a member of the Association. A title and abstract is to be sent in only when the author or co-author has the intention of presenting the paper.

*Publication of Abstracts.*—The abstracts will be published in the June number of the "Journal of Dairy Science." Please typewrite this plainly since the abstract is a copy for the printer. Leave ample margins and a double space between lines. Prepare the abstract so that it is ready for final publication.

*Length of Abstracts.*—The abstract must be short, 500 words or less.

*Contents of Abstracts.*—The abstract should contain concise statements of (1) the problem under investigation, (2) the experimental method used, and (3) the essential results obtained. Charts, graphs, photographs and tables will not be accepted.

*Time for Presentation of Papers.*—Ten minutes will be allowed for the presentation of each paper.

To be included in the program the title and abstract must be in the hands of the chairman of the Program Committee not later than April 15, 1940.

All communications relative to the program should be addressed to Dr. B. E. Horrall, Department of Dairy Husbandry, Purdue University, W. Lafayette, Indiana.

## PRESIDENT'S MESSAGE

The president's message to the members of the American Dairy Science Association should voice the opinions of the full Board of Directors. I trust that my associates will agree with my comments.

According to our secretary's report, which may be found in our Journal of August, 1939, the number of our members was above 1,400 with the addition of 149 affiliated members.

We hope that through the alertness and friendliness of our members, invitations to join our Association will be received by many good prospects. "Section 1. Any person is eligible to membership who is formally announced by an Agricultural College or Experiment Station, or by the Bureau of Dairy Industry of the United States Department of Agriculture or by the Canadian Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or any person fulfilling a position of responsibility connected with the dairy industry who has had a college or university training in technical science, or any person filling a responsible position in the dairy industry of a professional character requiring a technical knowledge of dairying of a high order."

The success of our Association depends on the number and the quality of our members. That is one of the reasons why student affiliation has been made possible at \$3.00 per year. This includes the subscription for our Journal. Student affiliation is open only to four-year and graduate students who are majoring in some branch of the dairy industry. Several student branches of the American Dairy Science Association have already been organized in dairy departments and it is urged that students and teachers in other departments consider a similar action. If it seems not wise to organize a student branch, or an affiliated group of students, it is desirable that the students receive the information that they individually may become student affiliates of our Association and receive our Journal for \$3.00. Also all students affiliated may become regular members after graduation on the payment of the usual \$5.00 fee, without contributing the additional \$5.00 initiation fee.

THE JOURNAL OF DAIRY SCIENCE has now reached the place in its development where it is the best publication that students in college, as well as older students in the industry, can read. It contains not only articles in science, but it presents abstracts of 68 foreign and domestic journals and 9 special publications as well. These concentrations are made possible by 99 abstractors who give freely of their time.

The readers as well as the contributors to our Journal will be interested in the effort that is being made to standardize the style of the manuscripts.

They, also, will be happy to know that an index of the first 20 volumes is being prepared.

Now may I call attention to the program of our next convention. Doctor B. E. Horrall, of the Department of Dairy Husbandry, Purdue University, is chairman of the program committee. His task can be made a pleasant and satisfactory one to himself and to our Association if our members will bear in mind the recommendations of the program and resolutions committees of this year. The committee that built the program gave us this statement. "The committee recommends that the Departments of Dairy Husbandry or Divisions from the various Experiment Stations exercise the same care in reviewing subject matter of the individuals presenting papers, as they would if such papers were to be presented in the most conservative scientific journals."

The resolutions committee offered the following improvement: "Therefore, be it resolved:

"That the members of the Association in the future exercise greater discretion in submitting titles and abstracts to the Program Committee when there is little likelihood of the authors' being able to attend the meeting, since it is believed that the abstracts should not be regarded merely as a convenient method of securing advance publication of research in progress."

Often I hear the statement: "I hardly know how I can arrange to go to the convention this year, but I just can't afford to stay away." We shall look for you at W. Lafayette the last week in June, 1940.

E. S. GUTHRIE,  
*President*

# JOURNAL OF DAIRY SCIENCE

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VOLUME XXIII

FEBRUARY, 1940

NUMBER 2

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## HYPERMAGNESEMIA WITHOUT CLINICAL SYMPTOMS IN DAIRY CATTLE

M. W. EVELETH, D. F. EVELETH AND F. E. WALSH

*Veterinary Research Institute and Department of Veterinary Obstetrics,  
Iowa State College, Ames, Iowa*

There are numerous reports in the literature dealing with hypomagnesemia in cattle. Most of the data on blood magnesium have been obtained in studying the metabolic upsets associated with certain types of malnutrition, parturition and deficiency diseases.

Duncan, Lightfoot and Huffman (1) have established 2.414 mg. per cent as the average for the Mg content of the plasma of dairy calves. The range of values encountered by these workers was 1.62-3.83 mg. per cent. The data show a slight increase in plasma magnesium in the older animals. Duncan and Huffman (2) found that during the period of from November through April there was very little change in the mean value but that during May and June there was a rapid decline. From July to November these authors found a steady increase in the plasma magnesium. Hayden (3) did not find significant variations in the serum Mg in a series of milk fever cases.

The early work on the hypomagnesemia of cattle has been recently reviewed by Allcroft and Green (4) in their report on the seasonal trends of the serum magnesium of a herd of Hereford cows maintained on pasture throughout the year. The data of these investigations show a definite peak for the serum in August while the lowest average value was found in December.

It is the purpose of this report to furnish evidence of a variation in the serum magnesium of a herd of Jersey cattle subjected to the influence of drought conditions.

### EXPERIMENTAL

The animals used in obtaining the data on which this report is based were all pure bred Jersey cattle. This herd was maintained for the commercial production of milk. Periodic examinations were made for disease control and for procuring the blood samples. The animals were adequately fed for milk production. All blood samples were obtained by venous puncture. The blood was then transported to the laboratory and the serum separated from the clot by centrifugation. A 1:5 dilution of the serum was made,

Received for publication June 14, 1939.

mixing 1 volume of serum with 2 volumes of distilled water and then adding 2 volumes of a 20 per cent solution of trichloroacetic acid. The proteins were removed by filtering through Whatman No. 42 paper. This filtrate is satisfactory for the determination of inorganic phosphate, acid soluble phosphorus, and the mineral bases. Inorganic phosphate was determined by a modification of the Fiske and Subbarow method (5). The calcium was determined by precipitating the calcium as the oxalate from 10 ml. of the protein free filtrate at a pH of approximately 6.0. This was accomplished by use of an ammonium hydroxide—ammonium oxalate mixture, 2 ml. of which just neutralized 10 ml. of the serum filtrate when methyl red was used as the indicator. The calcium oxalate was then washed and titrated with potassium permanganate.

Ten ml. of the calcium free filtrate corresponding to 1.66 ml. of serum were used for the determination of the magnesium. The data for 1934 were obtained by use of the Greenberg and Mackey (6) method while those of 1936 were obtained by the magnesium ammonium phosphate method described below. The magnesium ammonium phosphate was precipitated by adding 0.5 ml. of 10 per cent ammonium phosphate solution and 2 ml. of concentrated ammonium hydroxide to 10 ml. of the Ca free filtrate in a 15 ml. centrifuge tube. The tube was then stoppered and vigorously shaken. After a few minutes the stopper was removed to allow any fluid to run back into the tube and the tube again stoppered and left for 24 hours. The precipitate was then centrifuged down and the supernatent fluid discarded after which the tube was allowed to drain. The precipitate was then washed with 3 ml. of a 1:2 dilution of ammonium hydroxide. After the second centrifugation the tube was drained dry and the top wiped with a clean cloth. The phosphorus was then determined and the magnesium calculated from the phosphorus value. This method was checked against standard solutions of magnesium and compared with the Greenberg and Mackey method on blood filtrates with satisfactory results.

#### RESULTS

In table 1 are given the calcium, magnesium and inorganic phosphate averages for data obtained in 1934 and 1936.

TABLE 1  
*Calcium, magnesium and inorganic P of cattle serum*

No. animals	Mg. per cent Ca	Mg. per cent Mg	Mg. per cent Inorganic P
Jan. 1934 ...	6	9.54	3.12
Aug. 1934	34	11.14	4.33
Sept. 1934	59	10.12	3.18
Jan. 1936 ...	97	11.71	2.86
April 1936	99	10.31	2.81
July 1936	59	10.16	3.37
Sept. 1936	110	9.34	2.90
			6.50

These data show a definite increase in the summer values for the serum magnesium but offer no possible explanation. To rule out effects of age and

TABLE 2  
*Seasonal changes in serum magnesium of individual animals*

Cow No.	January		April		July		September	
	Mg. Per cent	Mos. Pregnant						
1	2.55	0	2.78	1	3.63	4	2.75	6
2	2.77	0	3.56	0	3.63	0	2.78	1
3	2.92	7	3.21	0	3.41	0	2.81	0
4	2.81	9	2.86	0	3.97	2	3.24	4
5	3.42	0	2.18	1	4.04	4	3.23	6
6	3.03	3	3.39	6	3.38	9	3.07	0
7	2.33	2	3.07	5	3.85	8	3.23	0
8	2.72	2	3.39	5	4.17	8	2.83	0
9	2.94	0	2.65	3	3.27	6	2.91	8
10	3.13	8	2.71	0	3.85	0	2.86	2
11	2.60	0	2.73	2	4.10	5	2.84	7
12	2.65	8	2.59	0	4.03	2	2.94	4
13	3.09	0	2.85	2	3.73	5	2.76	7
14	3.15	9	2.92	0	3.70	2	2.91	4
15	2.92	3	3.60	6	5.41	9	3.11	0
16	3.71	1	2.85	4	4.42	7	3.03	9
17	2.47	3	3.36	6	3.76	0	2.74	0
18	2.79	0	3.11	3	4.04	6	3.11	8
19	2.43	1	3.01	4	4.42	7	3.12	0
20	2.36	6	2.68	9	4.08	0	2.46	1
21	2.53	3	3.02	6	3.13	0	2.92	0
22	2.80	1	1.84	4	3.89	7	3.63	9
23	2.69	0	2.92	0	4.06	0	2.73	0
24	2.58	4	2.71	7	4.28	9	2.39	0
25	2.31	5	2.84	8	3.21	0	2.69	0
26	2.55	6	2.66	0	3.41	0	3.01	0
27	2.67	8	2.75	0	3.95	2	3.19	4
28	2.58	8	2.73	0	3.68	2	2.43	4
29	2.30	8	3.21	0	3.95	2	2.64	4
30	2.91	9	3.08	0	3.59	2	2.84	4
31	2.63	9	5.36	0	3.99	3	3.00	5
32	2.82	9	2.86	0	3.97	3	2.88	5
33	2.86	0	2.92	0	3.93	3	2.85	5
34	2.58	0	3.04	0	4.85	2	2.88	4
35	3.01	0	3.27	0	3.78	2	2.92	4
36	2.95	4	2.75	7	4.22	0	3.09	0
37	3.30	0	2.88	0	3.91	3	2.86	5
38	2.96	0	3.08	0	5.78	2	2.81	4
39	3.06	0	2.18	3	6.00	6	3.24	8
40	3.00	5	2.98	8	3.57	0	3.13	0
41	3.13	9	2.92	0	3.77	3	2.95	5
42	3.21	0	3.18	2	3.95	5	3.13	7
43	3.05	2	3.28	5	3.85	8	3.05	0
44	2.49	0	2.81	0	4.92	1	2.38	3
45	2.76	3	2.23	6	4.00	0	3.00	0
46	2.60	0	3.09	3	3.73	6	2.87	8
47	2.77	0	2.86	0	4.17	3	2.75	5
48	2.76	0	2.78	4	4.10	7	2.66	9
Av.	2.81		2.95		4.01		2.91	

gestation the serum Mg of 48 individual mature cows are shown in table 2. The magnesium value for the calendar month and the month of gestation, are shown in this table. Here the July value is much higher than any of the other three months.

#### DISCUSSION

The lack of any definite symptoms being associated with the hypermagnesemia does not suggest that the temporary high serum magnesium exerts a deleterious effect on the animal. No subsequent data have been obtained on the blood chemistry of these animals as a group but control data obtained on animals maintained at the Institute show that as long as the animals are eating fresh green grass this degree of hypermagnesemia does not develop. During the August 1934 period the grass was extremely dry and sparse. There were rains in August and September and fresh grass grew which the animals were grazing at the time the September samples were taken.

In 1936 the pastures were growing in September while they were very dry in July. Dry feed alone does not produce hypermagnesemia as cattle have been kept stabled and fed oats and alfalfa hay for long periods without causing an appreciable rise in the serum magnesium. Other herds have shown similar tendencies for wide variations in the serum magnesium from month to month.

A few preliminary experiments have been conducted to determine the effect of some salts on the serum magnesium of cattle and swine. In general, it has been found that sodium bicarbonate is very effective in raising the level of the serum magnesium. This may be a contributing factor in periods of drought when the feed is low in chlorides and phosphates. Just what the effect of a prolonged period of hypermagnesemia would be is problematical. The data in table 1 indicate that in these cases the calcium and magnesium in the serum varied independently of each other. Blood serum obtained from cattle in a moribund condition usually has both high calcium and high magnesium content. This appears to be an attempt on the part of the animal to compensate for an acidosis.

An interpretation of the significance of variations in the level of serum magnesium is impossible at this time. The association of tetany with hypomagnesemia is frequent. As far as could be observed the animals used in this study were normal. Information obtained in this investigation and data from other herds suggest that the level of magnesium in the blood stream is influenced by a large number of factors, most of which are of dietary origin.

#### SUMMARY

An increase in the level of the serum magnesium of dairy cattle has been found which is apparently due to drought conditions affecting the pasture.

There appear to be no clinical symptoms associated with a temporary hypermagnesemia in cattle.

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# COMPARATIVE PHYSIOLOGICAL RESPONSES OF DAIRY CALVES FED RATIONS HAVING DIFFERENT LEVELS OF MILK PROTEINS<sup>1</sup>

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The early recognition of the failure of milk alone to support life, promote growth and foster other normal physiological functions in calves evoked many theories and stimulated much investigation. Although there is considerable established information on the subject, much pertaining to the inadequacy of milk for calves remains in the controversial stage. Foremost in this field of theory, as previously discussed (19), is the etiology of disturbed mineral metabolism of milk fed calves. Sjollema (15) postulated that this anomaly is one of the symptoms of toxemia resulting from the consumption of excessive amounts of milk protein. The work reported herein was designed primarily to compare the responses of calves fed excessive amounts of milk proteins with reactions of other calves fed rations of normal nutritive ratio.

## EXPERIMENTAL

The experimental subjects and their grouping are indicated in table 1. The calves were paired, and subsequently one of a pair was allotted to each of the major groups, A and B. In this way it was possible to include the best and the poorest as well as the average in each group.

All calves received a basic ration of whole milk. The nutritive ratio of the ration of group A (control) was widened normally as specified in feeding standards (4), table 2. This was accomplished by feeding whole milk in adequate amounts to provide the prescribed amount of protein and by supplementing with sugar (glucose) in quantities sufficient to raise the total digestible nutrients to the required standard level. On the contrary, the nutritive ratio of the ration fed to group B was narrowed as the calves increased in weight. Milk was fed on the same quantitative basis as to group A, but the additional energy needed was provided by supplementing with commercial casein instead of glucose. Thus calves of both groups received the same amount of milk and approximately the same amount of total digestible nutrients per unit of body weight, but consumed decidedly different amounts of protein.

All calves received additional vitamins and minerals. The supplements added and the age of the calf when they were first introduced into the

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TABLE 1  
*Supplements added to the milk rations of individual calves in each group and duration of experimental treatment*

Group	Sub-group	No. of calf	Sex	Age when supplements were introduced				Age when removed	Cause for removal
				days	days	days	days		
A-	1	E-244	F	83	91	107	84	days 301	Experiment terminated
		E-248	F	41	49	65	65		Experiment terminated
	2	E-251	M	10	10	26	26	days 166	Slaughtered
		E-257	F	29	88	19	37		Died
B-	1	E-253	M	...	...	15	15*	days 176	Experiment terminated
		E-255	M	10	...	10	20		Experiment terminated
		E-245	F	69	77	93	...		Experiment terminated
	2	E-249	F	34	42	58	...	days 224	Experiment terminated
		E-252	M	9	9	25	70		Experiment terminated
		E-254	F	33	92	23	37		Died
	3	E-250	M	119	...	106	...	days 191	Experiment terminated
		E-260	M	10	...	9	41		Died
		E-246	F	148	118	118	92		Transferred

\* Honey.

TABLE 2

*Standard for daily amounts of protein, total digestible nutrients and mineral supplements*

Body weight	Digestible protein	Nutrients total	Nutritive ratio	Amounts of mineral supplements		
				Iron	Copper	Magnesium
<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>1:</i>	<i>mgs.</i>	<i>mgs.</i>	<i>gms.</i>
100	0.30	1.50	4.00	300	30	1.244
200	0.56	3.66	5.60	500	50	2.488
300	0.74	5.27	6.18	700	70	3.732
400	0.86	6.53	6.55	900	90	4.976
500	0.95	7.36	6.75	1050	105	6.220
600	1.00	7.90	6.90	1150	115	7.464

dietary regimen are shown in table 1. The quantitative basis of the daily mineral supplementation is indicated in table 2, the mineral data of which are based on previous observations (5, 19).

As indicated in table 1, several mineral supplements were omitted from the rations of calves in subgroups A-2 and B-2. All supplementary minerals and vitamins were withheld from the calf in subgroup B-3 until convulsions supervened. The purpose of these omissions was to determine the extent to which the absence of the supplements would accelerate the development of deficiency symptoms.

The organic supplements, glucose, honey and casein, were given at the evening feeding. The glucose was dissolved in warm milk, which combination was consumed readily. Feeding the casein involved somewhat greater difficulty, which increased as the calves grew older and received greater amounts of the supplement.

Care and management were as previously reported (19, 20). Blood samples were drawn bi-weekly and analyzed according to procedures already indicated (20). In addition, total nitrogen and non-protein nitrogen (nitrogen in trichloroacetic acid filtrates) of the plasma were determined by the semi-micro Kjeldahl method of Cavett (3).

#### EXPERIMENTAL RESULTS

The observations may be arbitrarily classified and considered under the topics of food consumption, growth, blood composition, health and autopsy.

*Food consumption*—The appetites of all the subjects were erratic from time to time. This was especially true of the calves receiving casein, which was somewhat unpalatable. The periodic intake of total nutrients, though variable for individuals, was essentially the same for the two groups as a whole. The intake of calcium, phosphorus and magnesium was slightly greater in group B than in group A due to the small amounts of these minerals in the casein. In addition to the prescribed dietary constituents the calves consumed foreign material including wood shavings, hair, gravel

and cinders. There was no marked difference in the nature and the magnitude of the depraved appetites.

*Rate of growth*—Considering the groups as a whole the difference in rate of growth was not marked. However, a study of the animals in pairs, as shown in figure 1, indicates with only one exception, E-252, that the casein-

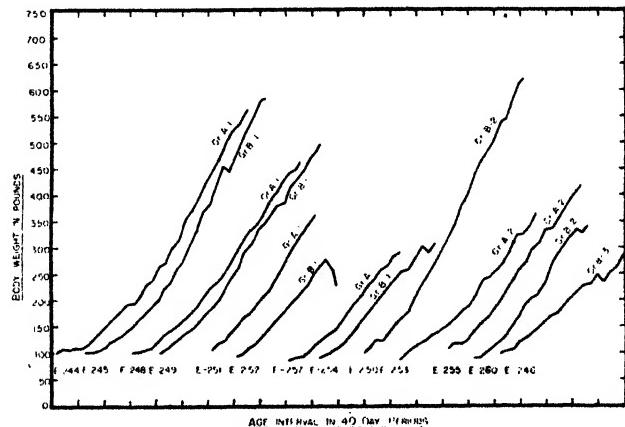


FIG. 1. Growth rate of individual calves.

fed calves grew at a rate either equal to or greater than the sugar-fed calves.

*Composition of blood*—As shown in figures 2 through 6, the most pronounced and the only significant difference observed in the composition of the blood of the two groups was in the nitrogenous constituents of the

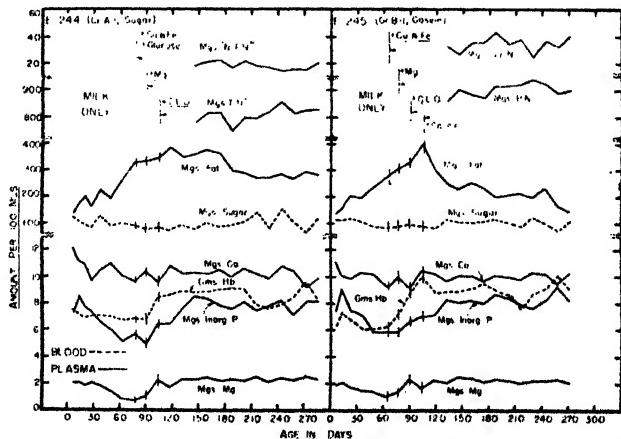


FIG. 2. Changes in composition of blood of calves, E-244 and E-245.

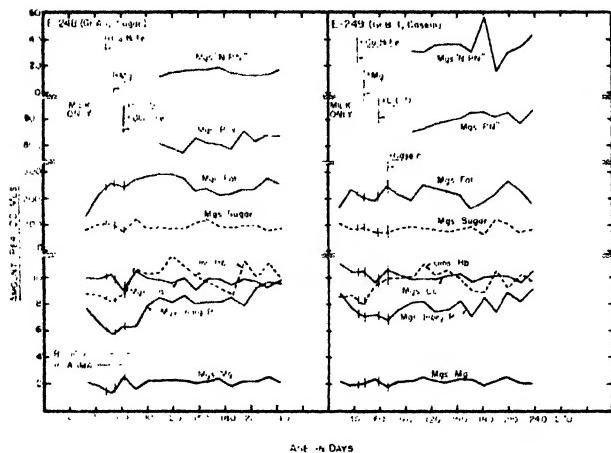


FIG. 3. Changes in composition of blood of calves, E-248 and E-249.

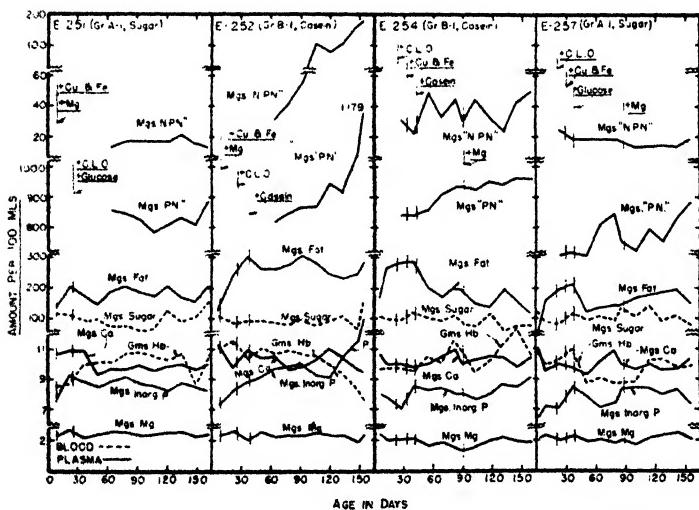


FIG. 4. Changes in composition of blood of calves, E-251, E-252, E-254 and E-257.

plasma. Both the non-protein nitrogen and the protein nitrogen (difference between the total nitrogen and the non-protein nitrogen) were higher and more variable in the casein fed calves than in the sugar fed calves.

Sugar of the blood was essentially the same for both groups. In accord with previous observations (19, 20) the values were relatively high.

The plasma fat was not strikingly different in the two groups. Yet a comparison in the last periods of the experiment shows that the trends of the plasma fat tended to diverge slightly due primarily to the decrease of

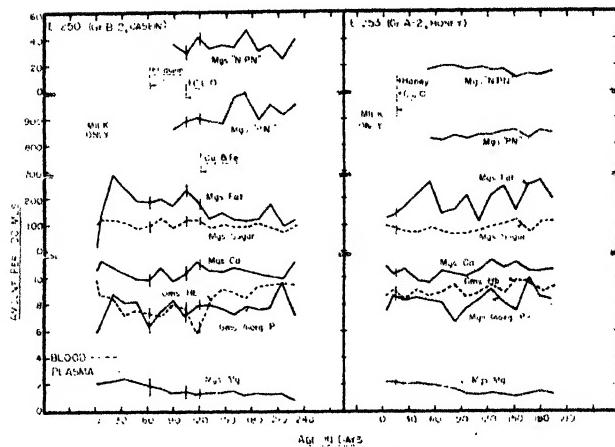


FIG. 5. Changes in composition of blood of calves, E-250 and E-253.

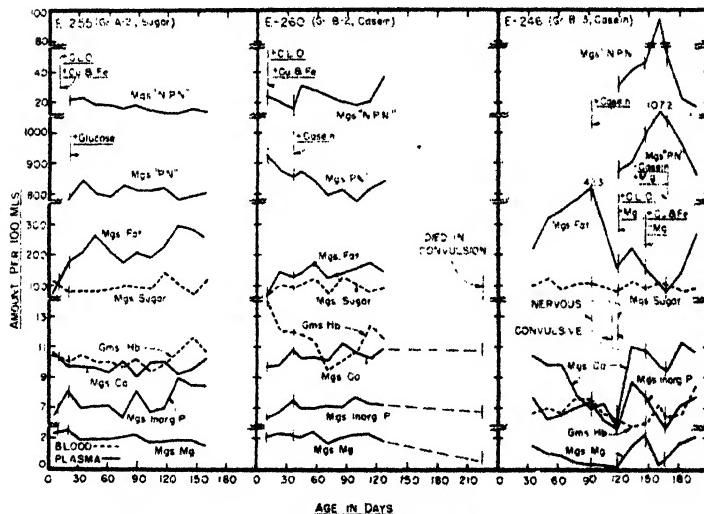


FIG. 6. Changes in composition of blood of calves, E-255, E-260 and E-246.

the level in group B and the maintenance or slight increase in the level of group A. Concordant with previous results (19, 20), modifications of the fat intake were manifested in variations of the plasma fat. However, the change of plasma fat was not always commensurate with the alteration of the quantity ingested.

There were no marked differences in the hemoglobin levels of groups A and B. As indicated in figure 5, honey apparently had sufficient minerals to maintain the hemoglobin well above the anemic level. The values in all

calves were rather variable especially during the summer when the temperature was high. Whether or not the amount of water consumed played an important rôle in the hemoglobin concentration is a matter of speculation.

Calcium, inorganic phosphorus and magnesium of the plasma revealed no significant differences in the corresponding subgroups of the two major groups. Excluding group B-3 the calcium and the inorganic phosphorus were within the normal range for all subjects except E-252, in which calf the inorganic phosphorus attained an abnormally high level in the last stages of life.

The plasma magnesium remained at a relatively uniform high level for all calves receiving the supplemental magnesium carbonate; whereas E-250 and E-253 (figure 5) receiving no magnesium supplement revealed definite downward trends during the early spring but very little change during the summer. Two younger calves, E-255 and E-260 (figure 6) started in the spring, maintained a relatively high level throughout the summer in spite of the absence of supplemental magnesium. However, in early fall there was a downward trend in both calves and one of these, E-260, later died in the throes of a violent tonic-clonic convulsion, which was accompanied by hypomagnesemia.

The changes in the composition of the blood of E-246 (figure 6), the only animal assigned to group B-3, merits individual consideration. Before casein was introduced into the ration, both calcium and magnesium showed a marked downward trend, which subsequently was not perceptibly altered by additional milk protein. The reduction continued until the onset of tetany, which was alleviated when the levels of calcium and magnesium were raised, respectively, by feeding cod liver oil and magnesium carbonate. After the restoration of plasma calcium and magnesium to the normal level, magnesium carbonate was withheld and copper and iron introduced to ascertain whether or not their presence would aid in the maintenance of the magnesium level. The resulting marked drop in the magnesium and the slight rise in hemoglobin precluded further investigation in this direction. The reintroduction of magnesium carbonate was followed by another rise in the plasma magnesium.

There were several changes in the composition of plasma of E-246 definitely associated with the introduction of casein into the ration, which dietary change also involved a reduction in the amount of milk fed. A decrease in the fat and an increase in the nitrogenous constituents followed. Nitrogen continued to rise until the consumption of casein decreased, resulting finally in total refusal of this supplement.

*Health and general well-being*—The general appearance as manifested in type and in state of flesh was essentially the same for both groups. In contrast, the calves receiving the high protein rations maintained a sleeker, smoother coat but manifested more sluggishness and lethargy than the

animals receiving the normal nutritive-ratio ration. Furthermore, the casein-fed group evinced symptoms of digestive disturbances more frequently and profoundly than the sugar-fed calves. Additional differences were manifested in the fecal material; that from the high protein ration was gelatinous or pasty in consistency and putrid of odor, but that from the sugar supplemented ration was liquid and almost odorless.

A peculiar action common to all was the tendency to contort the tongue. Whether or not this was symptomatic of a deficiency remains problematical.

Convulsions were observed in only two animals, E-246 and E-260, both of which were receiving additional casein but were also restricted in mineral supplements, table 1. The tetanic state in the case of E-246 was preceded for several weeks by nervousness, hyperexcitability and dysphagia; whereas in the case of E-260 there were no premonitory symptoms. Except for the violence of the seizures there were no distinguishing characteristics in the tetany of the two calves. In E-246 the convulsions were frequent but relatively mild, but in E-260 the first observed attack was very severe, terminating in death. The difference in age of the calves probably was an important factor affecting the relative severity of the seizures. In comparison with milk alone the additional casein did not seem to accelerate the onset of tetany.

The ante-mortem symptoms of E-257 (group A-1) and E-252 and E-254 (group B-1) may be summarized in the order of development: sluggishness, humped pose, anorexia, drawn abdomen, stiffness, sensitive muscles, dyspnea, apparent abdominal pains, nasal discharges, weakness, lassitude and finally death. There was no evidence of either nervousness or tetany.

*Post-mortem findings*—Gross examinations of E-252, E-254 and E-257 revealed marked gastritis, enteritis and slight peritonitis. Evidently the etiology was nutritional, either directly or indirectly. The constant presence of the rough undigestible foreign material in the alimentary canal is one factor that probably was responsible for the initiation and subsequent aggravation of the observed catarrhal condition of the mucous lining of the abomasum and the duodenum. Slight abnormalities of the other organs apparently were secondary.

In the case of E-260, the digestive organs appeared to be normal, but the heart was very hemorrhagic, lungs slightly congested, spleen hypertrophied, gall bladder thick and leathery, and the kidneys somewhat degenerated. Tissue cultures of the heart revealed bacterial infection. The extent to which the diet was involved in the etiology is uncertain.

#### DISCUSSION

The pre-supplementation trends of several mineral constituents of the blood of older calves used in this investigation and post-supplementation changes are in accord with previous observations (19, 20). The immediate

responses to the mineral supplements were the same for both major groups. However, nearly all the magnesium values are slightly low in comparison with the results of other investigators. Since it has been shown that the presence of manganese interferes with the magnesium determination, it is possible that manganese is partially responsible for some of the high magnesium values in the plasma of calves receiving this supplemental element in mineralized milk rations.

The presence of supplementary glucose in the rations of group A had no obvious effects on either the blood sugar level or any of the inorganic constituents of the plasma. Though changes in blood composition might have taken place, the time, 15 hours, elapsing between glucose ingestion and blood extraction evidently was sufficient to permit reestablishment of a normal equilibrium and balance.

As previously noted, the most striking difference between the blood constituents of the two groups, A and B, was the amount of nitrogenous constituents. The comparatively low levels of nitrogen in the plasma of the sugar fed calves may be attributed in part to two factors: first, a reduction of the nitrogenous constituents of the blood by a suppressive action of glucose (8, 11, 14) and second, the decrease in amount of protein consumed per unit of body weight.

The quantitative differences in protein intake appear to be the principal factor contributing to the differences in the level of nitrogenous constituents of the plasma. On a dry matter basis the amount of protein in the rations of group A ranged from 27.3 per cent for the young animals to 15.0 per cent for the older ones; whereas the corresponding range for group B was from 27.3 per cent to 54.0 per cent. The elevation of the nitrogen level of the blood with increased protein consumption is in accord with the observations of other investigators (1, 16, 17). However, it is interesting to note that the casein increased not only the non-protein nitrogen but also the so-called protein nitrogen of the plasma. The extent to which this increase was a direct result of the additional protein assimilated remains a moot question.

The exceedingly high values of the nitrogenous constituents observed in E-246 and E-252 cannot be ascribed entirely to the intake of protein. In the case of E-252 the highest values were attained several days before death when the intake was nil. These observations support the contention of Möllgaard as reported by Ritzman and Benedict (13) "that the physiological condition of the individual is a greater factor in protein utilization than the amount of protein ingested or even its character."

The high casein consumption changed not only the chemical composition of the blood but also altered the physical stability as manifested in the increased coagulability of the plasma when heated slightly and the greater tendency of the blood to clot when drawn.

In common with other animals calves need a relatively large proportion of protein in the diet in the early stages of life, the proportion needed presumably becoming less with advancement of age. Thus Sjollema (15) postulated that when older calves are restricted to a milk ration they are overfed with protein resulting in physiological disturbances involving alterations of the minerals of the serum. If this theory be true, it is reasonable to assume that the addition of proteins to a milk ration would greatly accelerate the onset of the disturbance of mineral metabolism. Contrary to this postulation, the feeding of excessive amounts of casein over a period of six months to calves of various ages apparently produced no marked changes in the mineral constituents of the plasma.

Furthermore, the only case in which degeneration of any organs could be associated with high protein rations is that of E-260. Since bacterial infection was also involved, the etiology remains complicated. Apparently the death of the other calves, E-252, E-254 (casein-fed) and E-257 (sugar-fed) was caused primarily by the presence of indigestible foreign material in the alimentary canal.

Though the results of this experiment are somewhat discordant with Sjollema's theory, it is probable that the quality of the protein as well as the quantity should be considered. This point of view is substantiated by the results from feeding certain proteins of vegetable origin. Calves fed milk rations supplemented with alfalfa hay, the average nutritive ratio of which is 1:3.7 in comparison with 1:3.9 for whole milk, revealed no evidence of metabolic disturbances (2, 9, 12, 18). On the contrary Kuhlman (7) reported that when cottonseed meal was suspended in milk and fed to young calves, death invariably followed without any associated evidence of tetany (6). When linseed oil meal was fed in a similar manner, death did not ensue but the animals became very nervous and excitable. Sjollema and Seckles (16) observed that the administration of large quantities of peanut meal or gluten of wheat to cows produced changes in the mineral constituents in the serum, which disturbance was accompanied by symptoms of intoxication.

That the nature of the protein plays an important rôle in tetany is further evidenced (10) by convulsions produced in dogs fed diets in which gliadin was the sole protein, furnishing 16 per cent or more of the caloric intake. Another diet containing casein to the extent of 28 per cent of the caloric intake never caused similar reactions.

The results as a whole do not indicate that milk proteins, casein particularly, are the primary etiological factor in the abnormal mineral metabolism observed in calves restricted to milk rations. Though in some cases high casein consumption was followed by abnormal mineral metabolism, evidence that the disturbance was not induced by the milk protein *per se* is deduced from a consideration of (a) the absence, in one case, of several

mineral and vitamin supplements from the diet previous to the disturbance and the subsequent remedial effects of these supplements, (b) the failure of additional casein consumption to accelerate perceptibly the onset of disturbances in mineral metabolism, (c) the inconsistent relationship between variations in nitrogen constituents and in mineral content of the plasma, (d) the pathology of the digestive tract, common to calves in both major groups and (e) complications from bacterial infections.

#### SUMMARY

1. The responses of two major groups of milk-fed calves, one receiving a ration of normal nutritive ratio and the other of narrow nutritive ratio, were compared.
2. All calves regardless of supplements possessed a depraved and erratic appetite.
3. The consumption of abnormally large amounts of casein by calves did not adversely affect growth or general appearance, but induced lethargy and occasionally digestive disturbances.
4. The most pronounced difference observed in the blood constituents of the two groups was in the plasma nitrogen, which was high in the calves receiving the protein-supplemented ration and comparatively low in the sugar-supplemented.
5. The results do not substantiate the postulate that milk proteins constitute the principal etiological factor in the disturbance of the mineral metabolism of milk-fed calves.

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## "NICKING" IN DAIRY CATTLE\*

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"Nicking" is a term sometimes used by breeders to convey the general idea that the results of some mating or group of matings were unexpectedly good. In such a case the sire and dam are said to "nick well." More rarely the term may be used to mean that the results were unexpectedly bad, in which case the mates may be said to have "nicked poorly" or "not to have nicked well." The term is rarely used in scientific writings because it lacks precision and because the situation it is intended to describe can have arisen from several very different causes.

In the first place an offspring much better than either parent can result merely from the part which chance plays in Mendelian segregation and recombination. The Mendelian laws of inheritance lead to the expectation that the most probable genotype of an offspring will be midway between the genotypes of its two parents, but that an individual offspring may vary widely in either direction from this expectation. Chance in Mendelian segregation and recombination may well be the most general cause of what breeders call "nicking" in cases which concern only one or a very few offspring. But because each gamete is an independent sample from the genotype of a parent, sampling errors tend to cancel each other in averages and can rarely cause several offspring all to deviate from expectation markedly in one direction.

A second genetic process which may lead to results breeders might describe as "nicking" is that the genotype of one or both parents may be far better (or worse) than is supposed. The correlation between genotype and phenotype is never perfect and for many economically important and physiologically complex characteristics it is apt to be low. A genotype is known only indirectly and imperfectly from the phenotype of the individual and from the phenotypes of its offspring, ancestors and collateral relatives. Occasionally an animal's genotype will really be much better than is estimated and the offspring from mating two such animals will delight the breeder so much that he may call it a case of "nicking" to express vividly his surprise at the result. This is apt to be the main cause of "nicking" where the case concerns a set of several full brothers and sisters. In such a case increasing the number of offspring from that one mating would

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\*\* Now with Louisiana Agricultural Experiment Station.

not tend to decrease the evidences of "nicking," as would happen with increasing number of offspring if the cause were merely an unusual result of chance in Mendelian segregation and recombination.

Errors in estimating the genotypes of parents would tend (if random) to cancel each other in estimating the average genotype of a number of parents, but not all errors in estimating genotypes are random. For example, a group of paternal half sisters are apt to be reared in the same herd under much the same conditions. If the special conditions which prevailed in that herd have affected producing ability more than is realized by the man who is estimating breeding values, the errors in his estimates will tend all to be biased in the plus direction (or all in the minus direction) and will not cancel each other very effectively with increases in the number of sisters involved. Errors in estimating genotypes from phenotypes may thus sometimes lead to "nicking," even when several parents are involved.

A third possible cause of nicking is that genes in certain combinations may have effects very different from their average effects. Deviations which other genes cause in the effect of a given gene are known technically as epistatic deviations or joint effects or non-additive combination effects. Illustrations of this sort of key-and-lock interaction are abundant in the field of mechanics, where the exact nature of each interaction can be seen. In fact the essence of a machine is that its parts are organized so that the machine as a whole can do things which its parts couldn't begin to do individually. Animals and plants are exceedingly complex machines and non-additive interactions of their genes perhaps may be as important among them as in deliberately designed but simpler machines like watches or automobiles. However plants and animals may have been prevented during their evolutionary history from incorporating into their gene systems much important interaction of this kind because the combinations of genes are re-shuffled (within the interbreeding population) with each Mendelian segregation and recombination and for the preservation of the species it has been necessary that a fairly large proportion of all the new combinations be able to function well enough to survive and reproduce. This would have led to heavy selection against genes which would produce good results in certain special combinations but bad results in most of the combinations in which the Mendelian reassortment would throw them. Such genes thus might be kept individually so rare that desirable combinations requiring the simultaneous presence of several of them would scarcely occur at all in such finitely limited populations as dairy breeds. *A priori* arguments can thus be adduced both for and against supposing that epistatic gene interactions are important. To determine the truth about this will require actual experiments or at least extensive observations properly interpreted.

The outward sign of "nicking" from this third cause would be that the merit of a sire proved on one group of females would be quite different

from his merit when proved on another group. Or if several sires were involved, their order of apparent merit when proved on females of one group might be quite different from their order when proved on another group. This could be an important occurrence if it happened often that the cows in two or more groups mated to a sire were each genetically much like the other cows within the same group but distinctly different from the cows in other groups. However there is no effective way of getting groups of cows that are genetically uniform within groups but that are contrasting from group to group, except by inbreeding in separate families more closely than is ordinarily practiced. Selection and assortive mating are almost powerless to achieve any appreciable group separation of this kind when the joint effect of two or more genes is involved, because the effect of one gene (*A*) cannot be entirely distinguished from the effect of another gene (*B*). It is thus doubly impossible to get one group homozygous for *A* and *b* and a contrasting group homozygous for *a* and *B*.

Actual families within a breed usually differ from each other only moderately and each has much genetic variability. Because of this, even simple two-factor interactions requiring only the joint presence of two genes like *A* and *B* might be widespread in the population and yet the families would rarely be genetically uniform enough or contrast with each other enough that such "nicking" would attract much attention. Joint effects requiring the simultaneous presence of three or more genes would be even less likely to attract attention.

These considerations make it seem unlikely that epistatic effects, even if they were known to be abundant and important in the population (and that is not clearly demonstrated although certain special cases are well known), would often be systematically biased enough to disturb sire proving seriously. Yet reports of cases where a sire was proved "good" on daughters of one bull but "poor" on daughters of some other bull, are heard from time to time among breeders.\*

The present article reports an investigation of the importance of "nicking" as expressed in the milk production and the butterfat percentage of dairy cows. The data used were collected for another purpose. The present method of testing for "nicking" has not hitherto been applied to this end, so far as we know. Perhaps it will be useful in other cases.

#### SOURCE OF DATA AND METHODS OF INVESTIGATION

The data used in proving thirteen dairy sires in Kansas Dairy Herd Improvement Associations were studied. These sires included seven Holsteins, three Jerseys, two Ayrshires, and one Guernsey. Each sire had at least two groups of daughters when the daughters were grouped according

\* See Heizer, E. E., et al. Nicking in dairy cattle. Proc. Amer. Soc. An. Prod. for 1938, pp. 67-72, for some cases of the kind that occasionally are found in writings on animal breeding.

to their maternal grandsires. Members of each group were thus at least three-quarter sisters (a few were full sisters) inasmuch as they had the same sire and their dams were all by another sire. The number varied from group to group. Each sire had at least two main groups of daughters and some of them had several smaller groups ranging in size down to only one daughter in a group. The methods of analysis weighted these inequalities in group size in such a way as to use all the information in the data without bias from variations in size of group.

All records used had been previously corrected to a mature equivalent basis by the Bureau of Dairy Industry. For bulls numbered 1 and 4 the records had been converted by the 70, 80 and 90 per cent basis, while records of the remaining bulls were corrected by the more recent Bureau of Dairy Industry factors. The high record of each daughter and of each dam were used for all bulls proved except in the case of bulls numbered 7 and 10, for which the average of all records were used.

Tables 1 and 2 show the analysis of variance\* pertaining to differences in milk production and in butterfat percentage between daughters within the various maternal grandsire groups and also between the means of those groups. This analysis merely asks of these data: Are the differences between these groups of daughters more than would be expected from the amount of variation found between daughters which are in the same group? Some group differences of this kind would be expected, even if all genes interacted additively, since it is hardly possible that all the various maternal grandsires would have been equal in their breeding values. The dams of some groups of daughters would have been higher producers than the dams of other groups. This difference in the productive levels of the groups of dams would need to be discounted. Only if the group differences between the daughters were unexplained by the differences in the records of their dams would there be evidence of "nicking." As one way of discounting the effects of differences between dams, an intermediate index (Index = twice daughter's record - dam's record) was figured for each daughter and dam pair concerned in proving the sire. Then the index thus derived was used in place of the daughter's record in an analysis otherwise just like that described in the preceding paragraph. This left the grouping the same as when the daughters' records were used. Again the variance found between the groups was compared to the variance found within the groups. The results are shown in tables 3 and 4.

There being some question as to whether in using the index in this way errors from Mendelian segregation, from effects of environment, etc., would be thrown equally into the mean square between groups and that within groups, a third method of analysis, which it was thought would

\* See Snedecor, G. W., 1938. Statistical methods applied to experiments in agriculture and biology. Collegiate Press, Inc., Ames, Iowa. See especially pp. 179-307.

TABLE I  
Milk production of daughters grouped by maternal grandmothers

Bull No.	Number daughters	Number of groups	*Mean milk production										F	
			Group number											
			1	2	3	4	5	6	7	8	9	10		
1	11	6	138	129	123	111	147	101					127	
2	14	4	105	92	82	99							329	
3	12	3	97										106	
4	15	3	115	102	76								283	
5	9	2	156	176									90	
6	28	10	147	130	120	149	120	137	126	184	108		308	
7	10	3	52	56	60								804	
8	19	9	124	118	129	154	125	102	144	120	136		169	
9	14	7	68	74	39	96	60	95					773	
10	22	4	62	69	58	52							756	
11	12	3	85	73	88								1171	
12	12	6	144	126	108	152	72	133					520	
13	22	7	126	145	125	116	119	163	154				2.25	

\* Ooded to nearest 100 pounds of milk.

\*\* P < .05.

Mean square between groups is greater than that within 9 out of 13 times.

TABLE 2  
Percent butterfat of daughters grouped by maternal grandmothers

Bull No.	Number of daughters	Number of groups	Mean fat percentage										F
			Group number										
			1	2	3	4	5	6	7	8	9	10	
1	11	6	3.22	3.44	3.42	3.76	3.46	3.60					3.44
2	14	4	4.30	3.94	4.21	4.04							0.0443
3	12	3	5.19	5.14	5.11								4.18
4	15	3	3.62	3.45	3.91								5.16
5	9	2	3.40	3.32	3.47	3.38	2.97	3.12	3.18	3.09	3.24		3.35
6	28	10	3.37	3.43	3.45	3.47	3.25	3.12		3.32			3.26
7	10	3	5.79	5.58	5.55	5.25							.0739
8	19	9	3.30	3.52	3.36	3.34	3.64	3.21	3.45	3.60	3.27		5.62
9	14	7	5.34	5.80	5.29	4.75	5.88	5.05	5.25				.2242
10	22	4	5.91	5.31	5.92	6.21							3.40
11	13	3	4.24	4.04	4.22								.0328
12	12	6	3.13	3.46	3.47	3.67	3.35	3.23					.1941
13	22	7	3.38	3.07	3.27	3.43	3.35	3.03	3.52				.4035
													5.86
													.0526
													.412
													.0944
													.0765
													.0300
													2.56
													.1175

Mean square between groups is greater than that within 6 times out of 13. \*\* P < .01.  
\* P < .05.

TABLE 3  
*Variation in indexes for milk*

Bull No.	Number of daughters	Number of groups	*Mean milk index										Mean square Between groups	Mean square Within groups	F			
			Group number															
			1	2	3	4	5	6	7	8	9	10						
1	11	6	118	145	136	108	187	82					133	1440	4653	3.23		
2	13	4	138	94	106	124							121	1373	730	1.88		
3	12	3	130	96	133								113	1807	1940	1.07		
4	15	3	98	36	31								77	6686	3218	2.08		
5	9	2	207	246									233	3068	5130	1.67		
6	28	10	130	136	130	163	125	117	140	248	115	174	141	3618	1977	1.83		
7	10	3	48	53	70								54	342	414	1.21		
8	19	9	142	132	139	184	145	58	94	111	139		132	2417	606	3.99**		
9	14	7	74	58	58	130	23		80	103			84	1545	1684	1.09		
10	22	4	72	68	60	55							65	266	611	2.30		
11	12	3	100	72	82								82	988	820	1.20		
12	12	6	161	140	102	163	56	115					130	2621	3974	1.52		
13	22	7	119	156	127	72	169	227	139				119	3741	5194	1.39		

Mean square between groups is greater than that within 5 out of 13 times.

\* Coded to nearest 100 pounds of milk.

TABLE 4  
Variation in indexes for butterfat percentage

Bull No.	Number of daughters	Number of groups	Mean index for per cent fat										F	Mean square
			1	2	3	4	5	6	7	8	9	10		
1	11	6	2.96	3.13	3.25	4.03	3.52	3.38					3.26	0.1827
2	14	4	4.60	3.72	4.82	4.22							4.48	.6121
3	12	3	5.19	5.30	5.68								5.28	.2809
4	15	3	3.70	3.55	4.52								3.71	.1002
5	9	2	3.64	3.46	3.46	2.54	3.31	2.92	2.58	3.50	3.40		.3804	.12461
6	28	10	3.55	3.57	3.47	3.38	2.54	3.31	2.92	3.50	3.40		.0660	.4427
7	10	3	5.44	5.35	5.35	3.42	3.94	3.53	3.99	3.82	3.12		.0634	.544
8	19	9	3.30	3.66	3.78	3.42	3.94	3.53	3.99	3.82	3.12		.362	.8310
9	14	7	4.88	6.26	5.30	3.61	6.81	5.19	5.41				.1461	.938*
10	22	4	6.18	6.14	5.60	6.65							.9125	.2161
11	12	3	3.90	4.02	4.59								.5.98	.1.48
12	12	6	2.98	3.44	3.54	3.90	3.12	3.17					4.03	.9125
13	22	7	3.60	2.74	3.20	3.67	3.38	2.80	3.76				3.30	.2432
													3.48	.1.26
													.2713	.3628

Mean square between groups is greater than that within 4 out of 13 times.  
\*\* P < .05.

\*P < .01.

minimize the effects of these errors, was tried. It consisted of subtracting from the variance that part which could be explained by the linear regression of the daughter's record on her dam's record. (For an example of this method, see pages 249-252 and table 12.3 of Snedecor, 1938.) That analysis was carried out for the two sires having the most daughters and the most groups of daughters (sires number 6 and 8) but was not extended to the others as it seemed to give results not very different from those of the other two analyses. Table 5 summarizes the results obtained with this method.

TABLE 5

*Results of tests of significance between daughter groups of sires 6 and 8 adjusted for differences in production of dams*

Sire number	Mean square of adjusted data		F	Comparative F values*	
	For test of significance	Within groups (error)		From average of daughter's method	From index method
6 (milk)	921.56	494.35	1.86	2.25	1.83
(fat percent)	.0748	.0766	1.03	1.03	1.44
8 (milk)	401.62	134.00	3.00	3.21	3.99
(fat percent)	.0319	.0428	1.34	1.18	1.48

\* The F values listed for comparison are taken from Tables 1, 2, 3, and 4.

#### RESULTS AND DISCUSSION

The F values in table 1 show considerable variation. For example, bull number 1 had 11 daughters divided in 6 groups and the average milk production of these groups ranged only from 10,100 pounds to 13,800 pounds. The mean square (variance) within groups was 977 as compared to 329 between groups. A contrast is presented by the data concerning bull number 8. The means of the various groups of his daughters range from 10,800 to 18,400 pounds. The mean square between groups is 3.21 times as large as that within groups. The F value would be statistically significant ( $F = 3.07$  at the 5 per cent level) if it stood by itself but we have singled it out here for illustration after we knew that among 12 possibilities bull number 8 offered the most extreme contrast to bull number 1. Therefore, its significance is not definitely established. For 9 of the 13 bulls the mean square between groups exceeds the mean square within groups but the differences are erratic and are not large, except for the two bulls just discussed. Table 1 by itself merely hints at the existence of group differences without proving them and it does not begin to prove that they are larger than might be expected after allowing for the differences between the groups of dams.

When the intermediate index for milk production was used instead of the daughter's record, the range increased for most bulls.\* The mean square

\* This of course is to be expected, unless the records of daughter (D) and dam (M)

between groups only exceeded the mean square within groups 5 (instead of 9) out of 13 times. Again the ratios between the respective mean squares were the greatest for numbers 1 and 8 with these showing opposite trends. The evidence thus suggests that even the faint hint of group differences which table 1 seemed to show may all be explained by observed differences between the dams with little (if any) residue left to be ascribed to "nicking."

The butterfat percentage of the daughters, grouped according to their maternal grandsires, showed variations that appeared in certain cases to be rather large (table 2). For example, bull number 6 with 28 daughters and 10 groups had group averages ranging from 2.97 per cent to 3.48 per cent. Yet the mean square within groups was almost the same as that between groups. Numbers 3 and 5 showed unexpectedly uniform group averages, each bull having a variance within the groups which would be significantly larger than the variance between the group means if the evidence on them stood alone. For each of these sires the groups of daughters differed less than would be expected if each group of daughters were a random set from a single population. For only 6 out of the 13 sires was the variance between groups greater than that within groups. Evidence of definite differences between groups was lacking.

As with milk production, the use of the index for butterfat percentage widened the range (table 4) in the means of the maternal grandsire groups as compared to the range shown when the butterfat percentage of only the daughters was considered. In only 4 cases (sires 1, 2, 6, and 12) out of the 13 was the value of the mean square between the groups greater than that within groups. As with milk production the discounting of differences between dams, as far as the index accomplishes that, makes the evidence for group differences appear even scantier than it did when the daughters' actual records were used without regard to the records of their dams. There is no definite indication here of any epistatic "nicking" in butterfat percentage.

Theoretical considerations lead us to expect that even if non-additive interactions between genes were abundant and important, such outward evidences of "nicking" as were being sought here would not be conspicuous in populations bred as dairy cattle usually are. They might still be important in crosses between breeds or between inbred lines. Hence these data tell little about whether non-additive interactions of genes which affect milk production and fat percentage are rare or abundant and important. They do indicate (subject to the limitation that only a few sires were studied) that in proving dairy sires with the kind of data usually

were perfectly correlated. The formula for the index ( $I$ ) is:  $I = 2D - M$ . From the well-known formula for the variation of a difference it follows that

$$\sigma I = \sqrt{4\sigma_D^2 + \sigma_M^2 - 2r_{DM}\sigma D\sigma M}$$

which when  $\sigma D$  is equal to  $\sigma M$ , reduces to:

$$\sigma I = \sigma D \sqrt{5 - 4r_{DM}}$$

encountered, "nicking" is not often important enough that the pedigrees of the dams need consideration if the records of the dams are taken into account.

#### SUMMARY

The records used to prove 13 bulls in Kansas Dairy Herd Improvement Associations were examined by the analysis of variance to see whether there were significant differences among the daughters of each bull when the daughters were grouped according to their maternal grandsires.

For 9 bulls out of 13, the variance in milk production between groups exceeded the variance within groups.

The mean square for the sire's index for milk production was larger between groups than within groups in only 5 out of 13 cases.

In 6 out of 13 cases the mean square for butterfat percentage was larger between the groups than within the groups.

As concerns the sire index for butterfat percentage, the variance between groups exceeded the variance within groups in only 4 cases out of 13.

Using linear regression to correct daughters' records for differences in the records of their dams gave results similar to those secured by the other two methods.

The data from these 13 bulls gave no indication that "nicking" is generally important enough to need much attention when proving sires. Differences between groups could easily have been due to chance variation in the sample of inheritance transmitted by the bull, or to differences in the environments that affected the different groups of daughters when their records were made.



# CAROTENE AND VITAMIN A IN THE NUTRITION OF GROWING DAIRY CATTLE<sup>1</sup>

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Jones, Eckles and Palmer (10) were among the first investigators to demonstrate the necessity of vitamin A for the proper nutrition of dairy calves. Bechdel, Eckles, and Palmer (1), and Converse and Meigs (3, 4, 12) submitted further evidence that calves require carotene or vitamin A. Hart and Guilbert (9) observed vitamin A deficiency in range cattle under natural conditions. Guilbert and Hart (7) found that about twenty-nine micrograms of carotene as it exists in alfalfa hay per day per kilogram of body weight prevented or cured vitamin A deficiency in cattle. Guilbert, Miller, and Hughes (8) working with cattle, sheep, and swine confirmed the above findings and reported that 25 to 30 micrograms of carotene daily per kilogram body weight, or 6 to 8 micrograms daily of vitamin A was just sufficient to prevent night blindness. Kuhlman, Gallup, and Weaver (11) found a commercial preparation of carotene dissolved in cottonseed oil to be an effective source of vitamin A for calves. Soldatenkiv (15) stated that feeding carrots to calves apparently accelerated recovery from respiratory diseases and built up resistance to infection.

In connection with investigations at the Pennsylvania Experiment Station (5) calves have been observed to become blind and to exhibit other symptoms of vitamin A deficiency while receiving levels of carotene intake (from timothy hay) in excess of that stated by Guilbert and Hart (7). Further investigations were, therefore, undertaken with the object of securing additional information regarding the carotene requirements of growing dairy cattle and to study the utilization of carotene from different sources of feedstuffs.

## METHOD OF ESTIMATING THE CAROTENE CONTENT OF FEEDSTUFFS

For the determination of carotene in the feedstuffs the Struve modification of the Guilbert method (6) was used. This method differed from the

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Guilbert procedure in that half saturated methyl alcoholic-potassium hydroxide was used for the digestion and saponification, and also in that absolute methyl alcohol was added to the ethyl ether solution and then the ether evaporated off on a water bath at 65° C. instead of evaporated to dryness in vacuo as in the Guilbert method. The final petroleum ether solution of carotene was compared to a standard potassium dichromate solution.

In order to check on the accuracy of this method of carotene estimation, biological assays were also made on a number of samples of the hays. The results indicated a satisfactory agreement between the values obtained by the two methods of assay.

#### GROWTH OF CALVES RECEIVING DIFFERENT LEVELS OF CAROTENE INTAKE

The object of this phase of the investigation was to compare the effect of different levels of carotene intake on the growth and well-being of young calves. Grade Holstein calves (ten bulls and three heifers) were used as the experimental subjects. Mow-burned brown alfalfa hay containing only 1.3 micrograms of carotene and dehydrated alfalfa containing 65 micrograms of carotene per gram were used as sources of carotene. These hays were mixed in such proportions that mixtures containing 1.3, 4, 9, 17, and 65 micrograms of carotene per gram were available for the feeding tests.

The calves were housed in a modern barn which was equipped with a heating and ventilating system. This equipment made it possible to maintain fairly uniform experimental conditions throughout the winter months, and prevented barn temperatures lower than 28° F. During the summer months the calves were turned out in a dry lot for several hours each day. A concentrate mixture, made up of feeds of low vitamin A content, but containing 11.3 per cent digestible crude protein and 69.9 per cent total digestible nutrients was used. The constituents were wheat bran, "Banner" oat feed, barley, linseed oil meal, molasses, salt, and irradiated yeast. All calves were fed whole milk during the first three weeks on experiment, after which skimmilk was fed. The concentrate mixture was supplied to the animals according to their appetites. Water and salt were supplied ad libitum. The hay was kept before the calves at all times, but the animals were usually 40 to 50 days old before appreciable amounts were consumed.

The carotene contents of the various hay mixtures fed as well as gains in body weight of the respective animals are shown in figure 1. While gain in weight of the animals was the chief criterion used to determine adequacy of carotene intake, close observations were made for other symptoms of vitamin A deficiency. The carotene content of the blood serum was run at two-week intervals. The Palmer method as modified by Connor (2) and later by White and Gordon (16) was used for these blood carotene determinations.

## DATA

In order to conserve space, the data obtained in this phase of the investigation have been reduced to graphic form and are presented in figure 1.

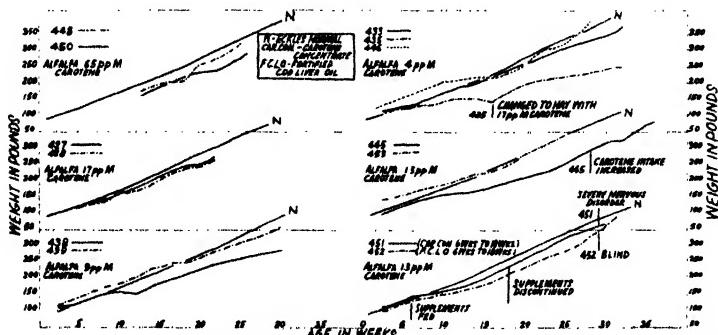


FIG. 1. Growth of calves while receiving equal quantities of hays of different carotene contents.

## DISCUSSION

Examination of the data presented in figure 1 show clearly that increases of carotene intake within the limits of this experiment did not result in increased growth of the calves. Even at levels of intake much below the reported minimum the growth continued normal for some time and was not greatly affected until the avitaminosis A reached an advanced stage. This may suggest that decreased growth rate frequently reported may be caused by intestinal, respiratory or other disturbances which so frequently accompany vitamin A deficiency. The average growth rate of two calves (Calves 448 and 450) which received the hay containing 65 micrograms of carotene per gram was no greater than the growth rate of calves (Calves 445 and 453) which received the hay containing only 1.3 micrograms, although this latter hay supplied only 4 to 8 micrograms of carotene per pound body weight per day. Several of the calves that received the carotene poor hay exhibited other symptoms of vitamin A deficiency. Distinct papillitis was observed in the eyes of calves 445 and 453 when they were several months of age. Calf 453 developed night blindness when approximately six months of age and calf 445 manifested several of the spasms which occasionally accompany vitamin A deficiency. Calf 452 was given from 0.6 to 0.8 gram of fortified cod liver oil daily and calf 451 was given from 0.6 to 0.8 gram of a carotene supplement for several months after they were placed on experiment. These supplements raised the vitamin A intake of the two calves to an equivalent of 10 to 12 micrograms of carotene per pound body weight per day. When these supplements were discontinued, calf 452 became permanently blind in about eleven weeks and calf 451 developed a severe nervous disorder. The administration of the carotene concentrate

at this time was not effective in promoting any noticeable improvement in the condition of calf 451 while calf 452 did show a slight improvement in general condition.

With the exception of calf 435 which did not consume much hay (0.6 pound per 100 pounds body weight per day), the hay with only 4 micrograms of carotene per gram was just as satisfactory as hays of higher carotene content in preventing vitamin A deficiency and in promoting growth. For these calves this hay supplied approximately twenty micrograms of carotene per pound live weight per day. No immediate benefit was observed as the result of feeding hays of higher carotene content.

Calves 445, 453, 451, and 452, that received the hay containing only 1.3 micrograms of carotene per gram, always maintained less than .01 milligram of carotene per 100 cc. of blood serum. When calves 451 and 452 received their respective vitamin A supplements, the blood serum contained .01 to .015 milligram carotene per 100 cc. serum. Calves 443 and 446 had blood serum values ranging from .01 to .02 milligram when fed hay containing 4 micrograms of carotene per gram. In the case of those calves fed hays with higher carotene content, the minimum blood value was .02 milligram per 100 cc. of serum. It is of interest to note that the blood serum value of calf 435 dropped to approximately .0025 milligram per 100 cc. When hay containing 17 micrograms per gram was fed, the blood carotene rose very rapidly to a level of .03 milligram per 100 cc. of serum. The blood serum of all calves usually dropped appreciably for the first few weeks after birth. After this period there was a general increase in blood serum carotene as the age advanced even at minimum levels of carotene feeding.

#### AVAILABILITY OF CAROTENE FROM DIFFERENT SOURCES

This phase of the investigation was designed with the view of determining the biological value of carotene from different plant sources. In these studies, twelve Holstein and seven Guernsey calves were used as experimental subjects. These animals were so distributed in the different experimental groups as to permit a comparison of the carotene requirements of the two breeds.

The concentrate mixture used was identical with that employed in the previous experiment. All but three of the calves received whole milk during the first few weeks of the experiment, after which they received skim-milk until approximately five months of age. Three calves, two Holstein and one Guernsey, were changed to skimmilk within a few days after birth. Two of these were supplied with a commercial carotene supplement and one calf was supplied with fortified cod liver oil. The following feedstuffs were used as sources of carotene: alfalfa hay, timothy hay, alfalfa molasses silage, corn silage, yellow corn meal, and carotene concentrate (carotene in cotton-seed oil). A fortified cod liver oil was also fed as a source of vitamin A.

Where the amount of hay fed was limited or where no hay was fed additional roughage was supplied to the calves in the form of beet pulp or wheat straw. An attempt was made to keep the calves growing and in the best possible state of health. All animals were weighed at weekly intervals and these weights were used in calculating the carotene and the food requirements of the respective animals for the succeeding week.

In these studies, changes in the fundus of the eye were used as a criterion of vitamin A deficiency. These changes were observed by means of an ophthalmoscope used according to the procedure reported by Moore (13, 14). Following a period in which the calves received a sub-minimal vitamin A intake, papillitis as well as other changes in the eyes appeared. These changes could be readily detected as they appeared but some difficulty was experienced in determining just when the calf was again receiving sufficient vitamin A. It had been observed in earlier work at this station (5) that the pupil of the calf's eye became dilated and lost its ability to contract on exposure to a bright light when the animal had been receiving a vitamin A deficient diet. When sufficient carotene or vitamin A was supplied to such calves, the pupil usually recovered its ability to contract. Therefore, in these studies, the dilation of the pupil was also used as a measure of vitamin A deficiency. In addition, the appearance of night blindness, digestive disturbances, respiratory disturbances, and the carotene of the blood were considered in evaluating results.

The calves were supplied carotene from one of the above mentioned sources at a definite level for several weeks. If no deficiency was noted the level of carotene intake was lowered progressively until a deficiency was observed. The level of carotene intake was then raised progressively until the deficiency symptoms disappeared.

#### RESULTS

The data obtained concerning the requirement of individual calves for carotene from the different sources have been summarized and are presented

TABLE 1

*Level of carotene intake above which no symptoms of vitamin A deficiency were observed*

Feedstuff	Micrograms per pound body weight daily	
	Holstein	Guernsey
Timothy hay	19	21
Alfalfa hay	14	15
Alfalfa molasses silage	33	...
Corn silage	17	...
Carotene concentrate	11	13
Yellow corn meal	18	...
Fortified cod liver oil* (vitamin A)	13	13
Fortified cod liver oil plus hay*	23	19

\* Calculated by assuming one U.S.P. unit equal to 0.6 gamma of carotene.

in table 1. The data are expressed as the number of micrograms of carotene per pound body weight per day required to cure or to prevent vitamin A deficiency. The micrograms of vitamin A from fortified cod liver oil are calculated from the manufacturers guaranteed minimum by assuming that one U.S.P. unit is equal to 0.6 *gamma* of carotene.

In table 2 the amounts of carotene in the blood serum of the above calves while receiving the various carotene intakes are presented.

TABLE 2  
*Blood serum carotene in relation to carotene intake*

Daily carotene intake***	Mean blood serum carotene**					
	5-10	10-15	15-20	20-30	30-50	50-400
<i>Source of carotene</i>						
Timothy hay .....	Trace	.005	.008	.015		
Alfalfa hay .....	Trace	.006	.008	.015	.03	.03-.22
Alfalfa molasses silage .....		.004	.014	.015	.01	
Corn silage .....		.005	.006	.008		
Carotene concentrate .....	Trace	.009				
Yellow corn meal .....			.014			
Fortified cod liver oil .....		.005	.01			
Fortified cod liver oil plus hay .....		.003	.005			
Average of all feeds .....	Trace	.0053	.008	.014	.02	.03-.22

\*\* Mgms. per 100 cc. serum.

\*\*\* Micrograms per pound body weight.

In eight cases (four Holsteins and four Guernseys) respiratory and intestinal disturbances appeared when calves were receiving sufficient carotene to prevent papillitis. The four Holstein calves made a favorable recovery when their carotene intakes were increased by only a few (5-15) micrograms daily. Two of the Guernsey calves also recovered completely when given increased amounts of carotene. On the other hand when the remaining two Guernsey calves were treated with Bisno-Pepsol compound, they failed to recover. An autopsy on one of the latter calves revealed a severe fibrinous pleurisy accompanied by a purulent exudate in the pleural cavity. In the more acute areas of inflammation numerous areas of emphysema were present. In the areas with a pneumonia of longer duration there were many abscesses. The intestines were full of food and did not appear to be inflamed although the calf manifested a severe case of diarrhea. It may be significant that at about the same time a number of pure bred calves in the College herd developed a similar type of intestinal and respiratory disorder when only a few days of age. A study of the rations of the dams of these calves indicated that a deficiency of carotene might be responsible. Following these observations a commercial carotene concentrate was administered to all calves during the first few weeks of their lives with favorable results.

The carotene requirements of some of the calves appeared to be slightly higher in cold than in mild or moderate weather. In the case of four Holstein calves and one Guernsey calf maintained at minimum levels of carotene intake, it was observed that these animals showed increased evidence of vitamin A deficiency following a cold spell and that marked improvement followed when the weather again moderated. More marked evidence of this increased requirement might have been observed had the experimental barn not been equipped with a heating and ventilating system which made it possible to maintain a temperature around 50° F. during the winter. The barn temperature never dropped below 28° F. and seldom went above 60° F. except in the summer months.

Observations made on some of the calves indicated the presence of a mild vitamin A deficiency between the time when the whole milk feeding was discontinued and when appreciable amounts of hay were consumed. One calf was supplied with the carotene supplement and another was supplied with fortified cod liver oil during this period. These calves showed some evidence of better health during this period than those that received no supplement. However, no permanent benefit from this supplementation was observed as the calves receiving hay alone seemed to be in about as good condition at the age of four months as those which had received the supplement.

#### DISCUSSION OF RESULTS

The data obtained in these studies reveal wide variations in the carotene intake necessary to protect different animals against vitamin A deficiency. The requirements of some animals appear to be much higher than others even when consuming similar rations. Some of the animals on timothy hay were apparently protected by a daily intake of 14 micrograms of carotene per pound body weight, whereas others required 19 micrograms. Similar results were obtained with other feedstuffs with the exception that as the biological value of the carotene in a feedstuff increases the range apparently becomes narrower. Similar variations were also observed in the amounts of carotene from different carriers necessary to prevent the symptoms of vitamin A deficiency. These results can be better understood by examining table 1 which summarizes these data. Of the various sources of vitamin A tested the carotene concentrate appears to be the most efficient source as levels of 11 micrograms of carotene protected all animals excepting one Guernsey. The fortified cod liver oil and the alfalfa hay were almost as efficient. With timothy hay as the carrier the requirements were appreciably higher, an intake of 19 micrograms being necessary for all the animals. The carotene of corn silage and of corn meal was utilized as efficiently as that of timothy hay. The requirement for the carotene from alfalfa molasses silage was much higher, a level of 33 micrograms being necessary for complete protection in all animals.

In any attempt to explain the above variations in experimental results, several possibilities suggest themselves. Apparently there is a wide variation in the ability of the animals to utilize the carotene from the same source. This being the case, one would also expect a wide variation in the ability of the animals to use the carotene from different sources. A study of the blood carotene values and a general study of the problem indicates this to be at least part of the explanation. It appears logical to assume that some of the substances in alfalfa silage measured as beta carotene were carotene degradation fractions or other related pigments that have no biological value. The data show that the carotene requirements from these sources were higher than from other carotene carriers. It is also conceivable that part of these pigments may have been carried into the blood and were measured there as carotene.

An unexpected result was obtained with the fortified cod liver oil, in that the requirement appeared to be lower when the oil was fed with little or no roughage than when it was fed with roughage. These results may have been caused by an increased rumination as well as other digestive activity following the feeding of roughage of poor quality. This increased rumination may have resulted in the destruction of part of the vitamin A of the cod liver oil before its passage into the absorptive mechanism of the body.

The data on blood carotene (table 2) indicate that when the serum carotene falls below 0.01 milligram per 100 cc. of serum marked symptoms of vitamin A deficiency are likely to develop at an early date. However, when the serum carotene drops below this value it is difficult to estimate carotene with a satisfactory degree of accuracy. When the value is 0.02 milligram or higher, the animal is usually receiving sufficient carotene for normal growth and well-being. Results obtained in treating intestinal and respiratory diseases indicate that the optimum requirement may be a few micrograms greater than indicated by the appearance of the eye. It also seems that a lowered vitality due to a vitamin A deficiency may have a bearing on the susceptibility of calves to scours and pneumonia. The results, however, show that intakes more than a few micrograms higher than indicated as sufficient by the appearance of the eye are of no further immediate use to the calf so far as growth is concerned. Higher intakes of carotene, on the other hand, may result in greater storage of vitamin A in the body tissues.

A comparison of the data on the two breeds (table 1) indicates that the Guernseys have a slightly higher carotene requirement than the Holsteins, but the difference between the breeds is much less than the differences between individuals within the breeds. In a few instances Holsteins appear to have the higher requirements, but the reverse is more often the case. When a fortified cod liver oil was used as the source of vitamin A, the vitamin A requirements of the Guernseys were no higher than those of the Holsteins.

## SUMMARY

The data obtained in this series of studies show that the minimum carotene requirement of growing calves is eleven micrograms per day per pound body weight. This level of carotene intake was sufficient to maintain growth and to prevent the usual vitamin A deficiency symptoms. The adequacy of this amount of carotene, however, depends upon the source of supply of carotene. While it appears desirable that the intake should be considerably above this amount it was surprising to find that increases above that level did not result in marked improvement in growth rate.

The order of availability of carotene to calves from the various sources studied is as follows: 1. Carotene concentrate, 2. Alfalfa hay, 3. Corn silage, 4. Corn meal, 5. Timothy hay, and 6. Alfalfa molasses silage.

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## STUDIES ON THE COMPOSITION OF BOVINE BLOOD. II. SEASONAL VARIATIONS IN THE LEVEL OF MAGNESIUM IN THE BLOOD PLASMA OF GROWING DAIRY CALVES

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In a recent paper (4) it was shown that the magnesium content of the blood plasma of growing dairy calves showed fairly close agreement from month to month and a definite tendency to increase from birth to eighteen months of age. The mean of 2,286 magnesium determinations was  $2.414 \pm 0.005$  mg. per 100 cc. of plasma. In connection with the foregoing statistical presentation it seemed of interest to rearrange these same observed values, without respect to age, into the respective calendar months in which they were taken and re-examine them with particular reference to the influence of the seasons of the year where the conditions of feeding and management were under control.

A survey of the literature discloses an ever-increasing interest in the close relationships between numerous processes in the animal body and the human organism with changes of season. That certain diseases occur more frequently in the transitional periods of spring and fall have been proved by statistics. Ritzman and Benedict (9), after a complete survey of the literature on standard metabolism experiments on steers and cows, came to the conclusion that some factors connected with season exert a dominant role in stimulating the metabolism of the tissues. The results of their own work with steers and cows have shown conclusively, that in every instance the basal metabolism dropped during the latter part of January and continued through March and that a high level of metabolism occurred during late May, June and July.

Weaver and Matthews (14) found that the fat test was highest during the first half of winter, gradually declined to the second half of summer, and then increased rapidly in the fall. The relations between the season of the year and the percentage of fat in milk have been reported by numerous other investigators and the literature has been summarized by Hays (5). They have all concurred in the opinion that regardless of when lactation begins, the percentage of fat followed a curve for the year, being lowest in June and July and gradually rising to the highest percentage during December and January. Regan and Richardson (8) found changes in the composition and the physico-chemical characteristics of milk when a state of positive heat balance was reached which probably resulted from blood

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changes instituted to facilitate heat disposal inasmuch as the blood is the ultimate source of the milk constituents. A close inverse correlation between the environmental temperature and the percentage of butterfat in cows milk was reported by Brooks (3).

Rosenkranz (10) observed that the thyroids of young cattle kept in stalls out of contact with sunshine exhibited a marked increase in parenchyma and a diminished number of follicles while cattle out on pasture, getting plenty of sunshine, had normal thyroids, well filled follicles and little parenchyma. Seidell and Fenger (11) reported that the average iodine content of the healthy thyroid gland of cattle was about three times as large during the months from June to November as during the months from December to May. R. H. van Gelder (13) found that the hemoglobin and red cells in the blood of Swiss mountain cattle increased in the summer but McCay (7) reported that the hemoglobin, iron and total phosphorus of the blood of milking cows was not influenced by summer sunshine or pasture. Kronacher and associates (6) state that the total solids of the blood of cows increased by about 10 per cent during the summer months. Suomalainen (12) made the interesting observation that during the month of January, when hedgehogs are in deep hibernation, the magnesium content of the serum increased markedly (from 3.20 mg. to 5.43 mg.) with a corresponding decrease in the ratio of calcium to magnesium.

The object of the present paper is to report results which add substantiation from a chemical and statistical standpoint to the cyclic influence of the astronomical seasons of the year on the amount of magnesium present in the blood plasma of the growing bovine. So far as we are aware the effect of the seasons on the changes in concentration of plasma magnesium of the growing calf has been nowhere reported.

#### MATERIALS AND METHODS

The basic data used in this paper are derived from experiments which were previously described so that a detailed statement of the general conditions of the experiment need not be repeated (4). For the present purposes, we have disregarded the effect of age on the level of magnesium in the blood plasma of the growing bovine and have regrouped and re-examined the same basic data according to the calendar months of the year. The pertinent features of the experiment may be summarized as follows: 1. during a period of three years, 2,286 magnesium determinations were made on the blood plasma of 107 normal dairy calves from birth to 18 months of age, 2. of the 107 calves, 47 were males and were removed from the experiment by the time they were six months of age, and 3. the calves received the following rations, depending upon their age, (a) whole milk, (b) skim milk, a mixture of corn and oats, alfalfa hay, (c) corn and oats, alfalfa hay, silage, and (d) pasture. All calves received the above standard rations fed in accordance to their

individual needs; therefore, under-nutrition and nutritional deficiencies were not involved in this investigation. The distribution of the birth dates are presented in table 1.

TABLE 1  
*Distribution of birth dates of 107 dairy calves by month*

Month	Number	Month	Number	Month	Number
Jan.	3	May	6	Sept.	9
Feb.	9	June	5	Oct.	19
March	15	July	10	Nov.	10
April	8	Aug.	10	Dec.	3

In order to investigate the influence of seasonal changes during the year, records of meteorological conditions were obtained from the U. S. Weather Bureau Station, East Lansing, for the years under consideration, together with some normal mean values for each month since the year 1864, and are summarized in table 2. The college is situated at longitude  $84^{\circ} 26' W.$ , and at latitude  $42^{\circ} 44' N.$ , and at an altitude of 863 feet above sea-level. It was thought that if meteorological factors did affect the concentration of plasma magnesium, a correlation between one or more of these factors and the level of magnesium might be evident.

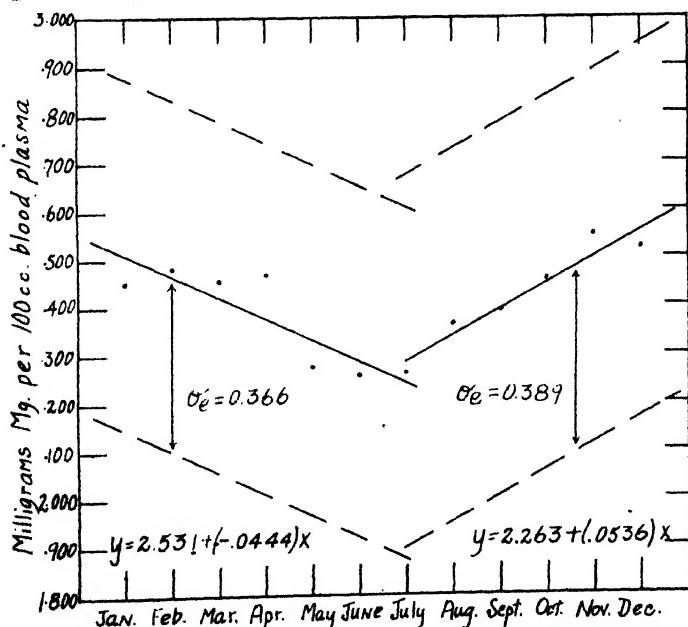


FIG. 1. Dots represent the mean monthly magnesium values of normal calves actually found and show the goodness of fit to the straight lines obtained from all of the experimental data in each six-month period by the method of least squares.

TABLE 2  
*Three-year composite meteorological data obtained by the U.S. Weather Bureau Station, East Lansing, and normal data  
 since the year 1861*

Temperature				Sunshine			Precipitation			Barometric pressure		Relative humidity	
Max.	Min.	Mean	Normal	Mean actual	Possible	Mean per day	Actual	Normal	Actual	Range	Mean	mm.	%
				Monthly								inches	mm.
°F.	°F.	°F.	°F.	hours	hours	hours	hours	hours	inches	inches	inches	mm.	%
Jan.	25.3	31.7	22.4	82.0	292.5	28	2.7	1.79	1.82	738.0	3.16	86	
Feb.	13.4	22.3	22.9	167.1	295.2	57	6.0	1.27	1.90	739.1	3.49	83	
Mar.	20.9	28.4	32.2	151.4	307.5	49	4.9	2.07	2.35	737.9	2.88	82	
Apr.	33.6	43.8	45.6	212.5	402.5	53	7.1	2.74	2.58	736.5	2.38	71	
May	47.2	58.5	56.9	302.3	454.7	67	9.8	3.44	3.42	737.8	1.75	67	
June	58.0	70.1	66.4	358.6	459.4	78	12.0	1.82	3.51	736.3	1.71	65	
July	60.0	72.0	70.9	372.3	464.0	80	12.0	2.34	3.10	737.4	1.79	62	
Aug.	56.6	68.3	68.5	312.7	430.7	73	10.0	2.45	2.82	738.0	1.57	67	
Sept.	52.9	62.9	61.4	198.8	374.8	53	10.0	3.54	2.91	738.5	1.69	76	
Oct.	40.0	49.0	50.3	123.1	341.9	46	4.0	3.31	2.47	738.8	1.97	80	
Nov.	29.2	36.3	37.5	59.5	292.1	31	3.0	2.28	2.48	739.1	2.87	85	
Dec.	18.6	25.7	27.2	69.5	280.8	25	2.2	1.99	2.07	739.5	3.06	90	

## RESULTS

The experimental data available for the present paper were reduced by common statistical methods and recorded in table 3 and supplemented with a graph (Fig. 1). The data presented in table 3 were derived from 2,286 determinations of magnesium in the blood plasma of normal dairy calves during the first 18 months of life and show the total number of determinations made in each calendar month during the period of investigation. The table also presents the mean magnesium value and its probable error, expressed as milligrams per 100 cc. of blood plasma, the minimum and maximum value, the standard deviation, probable error, and coefficient of variation for each calendar month.

The mean values in table 3 were derived from a frequency distribution table in which the data were classified into 23 classes with an interval of 0.10 mg. between each class. In figure 1, the dots represent the mean plasma magnesium values actually found and show the goodness of fit to the straight lines obtained from all the experimental data in each six-month period by the method of least squares. The computed limits of the band of normality (broken lines) for the first six months of the year are  $\pm 0.366$  mg. and  $\pm 0.389$  mg. for the last half of the year. The equations for both of the lines are also included in the graph.

TABLE 3

*The influence of season on the level of magnesium in the blood plasma of 107 normal dairy calves*

Month	Determi-nation	Mean	Min.	Max.	S. D.	P. E.	C. V.
							%
Jan.	195	2.451 $\pm$ 0.015	1.87	3.36	0.320	0.216	13.05
Feb.	193	2.481 $\pm$ 0.016	1.67	3.27	0.320	0.216	12.89
Mar.	190	2.455 $\pm$ 0.019	1.67	3.71	0.378	0.255	15.40
Apr.	191	2.470 $\pm$ 0.017	1.69	3.45	0.352	0.237	14.24
May	189	2.276 $\pm$ 0.017	1.67	3.45	0.344	0.232	15.11
June	178	2.258 $\pm$ 0.022	1.65	3.66	0.437	0.295	19.37
July	174	2.262 $\pm$ 0.019	1.62	3.65	0.380	0.256	16.80
Aug.	191	2.365 $\pm$ 0.019	1.70	3.63	0.395	0.266	16.70
Sept.	194	2.392 $\pm$ 0.019	1.63	3.60	0.399	0.269	16.68
Oct.	194	2.455 $\pm$ 0.018	1.82	3.71	0.380	0.256	15.46
Nov.	198	2.547 $\pm$ 0.017	1.90	3.74	0.363	0.245	14.25
Dec.	199	2.519 $\pm$ 0.017	1.84	3.83	0.363	0.245	14.40
Combined	2286	2.414 $\pm$ 0.005	1.62	3.83	0.378	0.255	15.65

In table 4 the results of the method of analysis of variance have been tabulated to show the significance of the difference between any two month means. The methods used for the determination of the standard error and the probable error in table 3 are of such a nature that they include all of the causes of variation. The method of the analysis of variance is based on the fact that the total variation is the result of various factors, some of which

are known and can be eliminated. In this case the variance due to between month means has been eliminated and the difference or the amount of variance which cannot be accounted for is assigned to the experimental error. The degrees of freedom for total sum of squares are equal to the total number of observations less one; the degrees of freedom for the sum of between month means are equal to the number of months less one; and the remaining degrees of freedom are the degrees of freedom for error.

TABLE 4

*Analysis of variance pertaining to the effect of season on plasma magnesium*

Source of variation	Degrees of freedom	Sums of squares	Variance	Experimental error
Total .....	2285	33,035.9484	14.4577	
Between month means .....	11	2,252.4309	204.7664**	3.679 class units
Within means .....	2274	30,783.5175	13.5372	.3679

\* Significant at the 1 per cent point.

t—table for between month means<sup>1</sup>

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Jan.	.....	.....	.....	.....	4.66	5.06	4.93	2.30	.....	.....	2.59	.....
Feb.	.....	.....	.....	.....	5.45	5.83	5.70	3.09	2.38	.....	2.46	.....
Mar.	.....	.....	.....	.....	4.74	5.13	5.00	2.39	.....	.....	2.06	.....
Apr.	.....	.....	.....	.....	5.14	5.53	5.39	2.79	2.08	.....	.....	.....
May	4.66	5.45	4.74	5.14	.....	.....	.....	2.36	3.09	4.76	7.24	6.50
June	5.06	5.83	5.13	5.53	.....	.....	.....	2.79	3.51	5.16	7.61	6.87
July	4.93	5.70	5.00	5.39	.....	.....	.....	2.67	3.38	5.02	7.46	6.74
Aug.	2.30	3.09	2.39	2.79	2.36	2.79	2.67	.....	.....	2.40	4.88	4.13
Sept.	2.38	.....	2.08	3.09	3.51	3.38	.....	.....	.....	.....	4.17	3.42
Oct.	.....	.....	.....	4.76	5.16	5.02	2.40	.....	.....	.....	2.48	.....
Nov.	2.59	.....	2.46	2.06	7.24	7.61	7.46	4.88	4.17	2.48	.....	.....
Dec.	.....	.....	.....	.....	6.50	6.87	6.74	4.13	3.42	.....	.....	.....

<sup>1</sup> Odds for various values of t.

$$2.0 = 21 : 1$$

$$3.0 = 369 : 1$$

$$4.0 = 15,772 : 1$$

$$2.5 = 80 : 1$$

$$3.5 = 2,149 : 1$$

$$4.5 = 147,058 : 1$$

For the statistical interpretation of the problem, the significance of the difference between any two month means can be found by noting the ratio of these differences to the standard error of these differences which have been computed by using the experimental error as the estimate of the standard deviation of the parent from which these samples came. If the ratio was such that it indicated significant difference, the value was recorded in table 4. The resulting t values are listed for the 5 per cent level of significance. Values greater than 2.576 are highly significant for the 1 per cent level, therefore, any ratio greater than 2.576 indicates a highly significant difference between month means. From an inspection of this table the statistical significance between any two month means can be evaluated by noting the size of the number which lies at the intersection of any two given

months. For example, it can be readily seen that the means of January and February are not significantly different from each other, but upon further inspection the number 4.66 lies at the intersection of January and May. The size of the number indicates the significance of the difference between the mean of January and the mean of May. At the bottom of the table a few values have been included to indicate the odds against the occurrence of such a difference due to chance. The value 4.66 indicates, therefore, that there is only one chance in over 147,058 times of this difference not being real. It is of interest to point out that the magnesium values for the months of May to August, inclusive, are significantly lower than the other months of the year, whereas the value for November is significantly higher than the other months of the year except December and February.

#### DISCUSSION

From an examination of the results obtained by the repeated determination of magnesium in the blood plasma of growing dairy calves and recorded in table 3, it is evident that the amounts of this element in the blood during the months of May to September, inclusive, were definitely lower than in the other months of the year. It is also of importance to point out that the magnesium dropped abruptly during May and continued to decline to lower levels during June and July before the trend was reversed and the concentration gradually increased to a maximum in November. These changes are marked in view of the fact that all of the mean values for the months of May to September, inclusive, are below the mean value for all observations ( $2.414 \pm 0.005$  mg.), and that all of the mean values for the months of November to April, inclusive, are above the mean value for all observations. The magnesium values during the fall and winter months were distinctly less variable than the values during the spring and summer months both in respect to standard deviation and coefficient of variation and undoubtedly reflect the influence of the same factor or factors which determined the decline in the mean. The greatest variability of standard deviation and coefficient of variation occurred during June.

Allcroft and Green (1), however, found that Hereford cattle running on pasture all the year round showed a seasonal variation in serum magnesium from a maximum in August to a minimum in December. Their winter values were frequently less than one-half of the summer values, the extreme range being from 2.8 mg. to 0.5 mg. per 100 cc. The addition of magnesium oxide to the mineral mixture appeared to alleviate the acuteness of the fall in serum magnesium from October to December but did not abolish it entirely.

The straight lines in figure 1, which have been obtained from all of the experimental data in each six-month period by the method of least squares, illustrate the general path of plasma magnesium throughout the calendar

year. This decline and increase in plasma magnesium is particularly striking in view of the fact that some of the values were obtained from calves which did not have access to sunlight. The results are also especially significant because they were obtained from calves offered a variety of rations, at all seasons of the year, so that the fluctuations observed in the blood magnesium are not referable to seasonal changes in the ration. The distribution of birth dates shown in table 1 also indicates that all types of rations were being fed. The abrupt drop in plasma magnesium during May occurred irrespective of the type of ration that the calves were receiving and irrespective of the age of the calves and was much more pronounced than the upward trend during the summer and fall months. An examination of the general trend of the curve suggests a striking parallelism in the concentration of magnesium to changes in season. Periods of high and periods of low concentrations not attributable to age or to the ingestion of food are definitely indicated by the curve.

When the mean monthly magnesium values obtained in this investigation were plotted against the monthly composited meteorological values obtained by the U. S. Weather Bureau, it was found that an inverse linear relationship existed between the plasma magnesium in the growing bovine and the mean temperature, actual hours of sunshine and rainfall, respectively. A direct linear correlation, however, was found to exist between the magnesium values and the values for barometric pressure and relative humidity. Thus a comparison of the meteorological values in table 2 and the mean monthly magnesium values in table 3 show increases and decreases concomitant with sustained changes in meteorological conditions. In general, the magnesium level was lowest during those months in which there were more than eight hours of sunshine per day and when the mean and maximum mean temperatures were above 55° and 70° F., respectively. Furthermore, the data show that relatively larger depressant effects were obtained during the months of May, June and July when the mean monthly temperatures and actual hours of sunshine were approaching their meridian. Armsby (2) placed the critical temperature of ruminants at approximately 56° F., while Hays (5) gave 70° F. as the temperature above which the increased metabolism is sufficient to influence mammary secretion. Regan and Richardson (8), however, found that there was a uniform increase in the respiration rate and that at 80° or 85° F., a pyrexial point was reached where the cows were no longer able to maintain heat balance. They also report that humidity and air movement are relatively unimportant for the comfort of the cow.

The results of this study support previous observations on both growing calves and mature cows and suggest that certain meteorological influences, such as sustained increases in temperature, humidity and low barometric pressure, are associated with manifestations of uneasiness or nervousness and capricious appetites. These symptoms are especially noticeable during

the spring and early summer months when the calves have to adjust themselves rapidly to new weather conditions. The relative humidity does not seem to be as intimately associated with the discomfort or uneasiness of the animals as the absolute humidity may be since the capacity of the air for water vapor is nearly doubled with each increment of 18° F. rise in temperature. Cyclic manifestations of physiologic activity are known to be associated in some unexplained manner with changes in season but it should be emphasized, however, that other factors besides meteorological changes are probably involved in the production of a seasonal drift in plasma magnesium, possibly through the influence of the endocrine or autonomic nervous system. So little is known of the causes and significance of changes in level of magnesium that these data are presented for record and without any particular attempt at interpretation.

#### SUMMARY AND CONCLUSIONS

The results of this study, based on the repeated determination of plasma magnesium in 2,286 samples of blood from 107 calves, show the changes in concentration of magnesium for the various calendar months of the year and the marked seasonal drop that occurred during the transition from winter to summer conditions when the calves had to adjust themselves rapidly to new weather conditions.

A number of direct environmental factors, the temperature, sunshine, precipitation, barometric pressure and relative humidity, have been considered and linear correlations have been indicated to show some apparent relationships with plasma magnesium but none have been found wholly satisfactory in explaining why these alterations occur in the animal body. Lower plasma magnesium values were associated with those months during which there were more than eight hours of sunshine per day and when the mean and maximum mean temperatures were above 55° and 70° F., respectively.

The changes in concentration cannot be ascribed to the effect of solar radiation because the magnesium values obtained from the young calves which did not have access to direct sunlight responded in the same manner as those which were turned out to pasture.

The results of the study suggest a striking parallelism in the concentration of magnesium to changes in season. Periods of high and periods of low concentrations not attributable to age or to the ingestion of food are definitely indicated.

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## MEASURING THE QUALITY OF ICE CREAM<sup>1</sup>

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There was a time when the public was the sole judge of the quality of ice cream. If the ice cream tasted good and had a smooth texture, it was acceptable regardless of the conditions under which it was made.

Public health officials now are giving more attention to the wholesomeness and sanitary quality of ice cream. One of the first attempts to improve the sanitary quality of ice cream was the establishment of bacterial standards. More recently the determination of *Escherichia-Aerobacter* organisms and the use of the phosphatase test have been advocated. Most states now have minimum butterfat standards and many states have adopted minimum weight standards for ice cream.

The annual ice cream survey conducted by the Kansas State Dairy Commissioner presented an opportunity to study several of the commonly used quality tests when applied to a large number of ice cream samples. The following tests were used: the standard plate count, tests to determine the presence of *Escherichia-Aerobacter* organisms, the phosphatase test, butterfat content, weight per gallon, and the score of the ice cream. The results obtained have been tabulated and an attempt has been made to evaluate each test individually and when used in combination with other tests as measures of ice cream quality.

### METHODS

During July, 1938, 318 samples of ice cream were collected from approximately 300 manufacturers. If available, a pint factory-filled package of vanilla ice cream was obtained, otherwise a hand-dipped pint package was taken. Samples were usually taken at the point of manufacture. Ice creams from large and small wholesale manufacturers, counter-freezer operators, tub-freezer operators and retail stores were examined in this survey. After each sample was obtained it was placed immediately in an insulated jacket and refrigerated with dry ice until delivered to the laboratory.

A standard plate count, using the methods outlined in "Standard Methods of Milk Analysis," 6th edition (1), was made on each sample. Numbers of *Escherichia-Aerobacter* organisms were determined by the tentative method outlined in "Standard Methods of Milk Analysis," 6th edition, duplicate tubes of brilliant green lactose peptone bile broth being inoculated with each of the appropriate dilutions of sample. Each positive presumptive test was confirmed by making a streak culture on eosin-methylene blue

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agar, from which a typical colony was isolated for determination of its behavior toward the Gram stain and of its ability to ferment lactose with the production of acid and gas. The results of the determination of *Escherichia-Aerobacter* organisms were expressed as the smallest quantity of sample giving a positive test for members of this group, because the use of only two tubes of each dilution did not justify a more definite statement of the results.

The efficiency of pasteurization was evaluated on the basis of results obtained with the New York field test for phosphatase. The published procedure as recommended by Scharer (2) was followed throughout. Control tests, in which water buffered to a pH of 9.6 was substituted for the buffered substrate solution, were run on all samples which gave positive color reactions. A sample was evaluated as under-pasteurized only when the amount of color developed in the regular test exceeded that in the control. In this way, possible misinterpretation of the results due to residual phenols or other interfering substances was avoided.

Two hundred and three samples were tested for butterfat using the Minnesota method (3), and 116 were tested for fat by the Garrett-Overman method (4). These two methods gave essentially the same results when compared on a series of 28 ice cream samples from this survey.

Weight per gallon of ice cream for each sample was calculated by weighing each container to the nearest gram before any of its contents were removed, and again after cleaning and drying the empty container.

The system used in scoring the ice cream was that used in the Students' National Contest in Judging Dairy Products (5). Fifty points were allowed for flavor, 25 for body and texture, 5 for color and package and 20 for bacterial counts.

#### RESULTS

*Standard plate counts:* The distribution of the standard plate counts for all of the samples, as well as for the samples obtained from each type of manufacturer, is shown in table 1. The standard plate count on 59.8 per cent of all the samples was 100,000 or less per ml. and 16.5 per cent had plate counts of more than one million. For the wholesale manufacturers, 54.5 per cent of the samples had a plate count of 100,000 or less, whereas 61.6 per cent of the samples from counter-freezer operators were in that group. Of the counter-freezer manufacturers, 25.1 per cent produced ice cream with counts of 10,000 or less, whereas the product of only 10.0 per cent of the wholesale manufacturers was in this class. In the count ranges from 50,000 to 100,000 and from 100,001 to 200,000 there were considerably smaller percentages of counter-freezer samples than of wholesale manufacturers samples, but the percentage of counter-freezer samples in the range above ten million was twice as great as the percentage of wholesale manufacturers' samples. Many of the counter-freezer operators were buying mix

TABLE I  
*Distribution of standard plate counts for all samples and for samples from each type of manufacturer*

Range of count per ml.	All samples		Wholesale manufacturers		Counter-freezer manufacturers		Tub-freezer manufacturers		Retail store samples	
	No.	%	No.	% of group	No.	% of group	No.	% of group	No.	% of group
10,000 or less	61	19.5	9	10.0	51	25.1	0	0	1	59
10,001 to 50,000	86	27.0	24	26.7	54	26.6	2	25.0	6	35.3
50,001 to 100,000	39	12.3	16	17.8	20	9.9	1	12.5	2	11.8
100,001 to 200,000	39	12.2	16	17.8	22	10.8	0	0	1	5.9
200,001 to 500,000	22	7.0	6	6.7	12	5.9	0	0	4	23.5
500,001 to 1,000,000	18	5.5	5	5.5	12	5.9	1	12.5	0	0
1,000,001 to 10,000,000	41	12.5	12	13.3	23	11.3	3	37.5	3	17.6
Over 10,000,000	12	4.0	2	2.2	9	4.4	1	12.5	0	0
Total samples	318		90		20.3		8		17	
% of all samples	100		28.3		63.8		2.5		5.3	

from firms which manufactured a product of uniformly low bacterial count, and this fact probably accounted for the greater percentage of samples with low counts in this group. The number of samples from tub-freezer manufacturers and retail stores was not sufficiently large to permit the drawing of definite conclusions. Four of the 8 samples from tub-freezer manufacturers, however, had standard plate counts above one million per ml.

Results from many laboratories and the experiences of many manufacturers have shown that the manufacture of ice cream with a standard plate count consistently below 100,000 per ml. can be accomplished by the use of ordinary precautions. Counts in the high hundred thousands and in the millions, as found in numerous instances in this survey, would indicate that either the quality of the raw materials or the methods used in processing were not satisfactory.

*Relation of standard plate count to score:* The ice cream samples were classified on the basis of standard plate counts, and total score minus the bacterial score, in order to determine if any relationship existed between standard plate counts and the quality of the ice cream as judged by other criteria. The results of this grouping of samples is presented in table 2. The ranges of scores used in making these groupings represent what might be termed excellent, good, fair, poor, and very poor quality. Approximately half of the samples in each bacterial range were classed as fair, scoring between 65-67.9, and in this range of scores there seemed to be no relationship between the standard plate count and the score. Of the 225 samples with counts of 200,000 or less per ml., only 20 scored below 65 and were thus graded poor or very poor. Eighteen of the 21 samples scoring 71 or more had a count of 100,000 or less per ml., and 76 of the 97 samples scoring from 68 to 70.9 had a count of 200,000 or less per ml. Although there was a marked tendency for good bacteriological quality to parallel high quality as determined by the criteria used in judging, there were occasional samples

TABLE 2  
*Comparison of standard plate count and total score minus bacterial score*

Standard plate count	Number of samples scoring					Total samples
	71 or more (Excellent)	68-70.9 (Good)	65-67.9 (Fair)	62-64.9 (Poor)	Less than 62 (Very poor)	
10,000 or less .....	5	24	28	4	0	61
10,001- 50,000 .....	11	26	39	7	3	86
50,001- 100,000 .....	2	9	22	5	1	39
100,001- 200,000 .....	0	17	16	3	3	39
200,001- 500,000 .....	1	5	9	5	2	22
500,001- 1,000,000 .....	1	4	10	2	1	18
1,000,001-10,000,000 .....	1	9	20	7	4	41
Over 10,000,000 .....	0	3	6	3	0	12
Totals .....	21	97	150	36	14	318

of good bacteriological quality which were poor or very poor on the basis of total score minus bacterial score. This is to be expected, since many defects such as feed, storage and oxidized flavors are not of bacterial nature and their incidence would not be expected to be correlated with bacterial counts. The organisms responsible for some bacterial defects are destroyed by pasteurization, yet the defect which they cause is unaffected and could reduce the flavor score of the ice cream.

When the count was above 200,000 per ml., approximately the same number of samples was scored above 68 as was scored below 65. Three of the 21 samples scoring 71 or more, and 21 of the 97 samples scoring from 68 to 70.9, had counts in excess of 200,000 per ml., and the counts on some of these were well above 1,000,000 per ml., indicating that a sample can be satisfactory from other standpoints and still be of poor bacteriological quality. Some of these cases may have been the result of the presence of thermoduric or thermophilic organisms which survived pasteurization but did not affect the flavor of the finished product. The results indicate that some correlation exists between low bacterial count and high total score minus bacterial score. This relationship failed to hold when the plate count exceeded approximately 200,000 per ml.

*Occurrence of Escherichia-Aerobacter organisms:* The distribution of the samples on the basis of the smallest quantity containing members of the *Escherichia-Aerobacter* group and the relationship between the numbers of these organisms and the standard plate count for the last 270 of the 318 sample are shown in table 3. *Escherichia-Aerobacter* organisms were absent in 1 ml. quantities of ice cream in only 37 instances, and were present in quantities as small as 0.001 ml. in 58 instances, indicating that some contamination of most of the samples occurred following pasteurization, since the phosphatase tests were negative in most cases. Such contamination was quite heavy in many instances.

The logarithmic average of the standard plate counts of the samples in each of the classes, based upon the smallest amount of ice cream containing the test organisms, increased progressively as the number of test organisms increased. A definite trend toward relationship between number of *Escherichia-Aerobacter* organisms and standard plate count was noted when individual samples were considered. Most of the samples with low counts contained few of the test organisms, and most of those with high counts contained the test organisms in considerable numbers. Apparently the factors responsible for the presence of numbers of *Escherichia-Aerobacter* organisms in ice cream also determine to quite an extent the total numbers of bacteria present, although there are numerous instances where other factors intervene and provide exceptions to this general relationship. As has been indicated previously (6), an ice cream sample which is negative to the phosphatase test, but which contains *Escherichia-Aerobacter* organ-

TABLE 3  
*Relationship between the standard plate counts and the smallest amount of ice cream containing *Escherichia-Aerobacter* organisms*

Smallest amount containing E.-A. organisms	Samples in class	Logarithmic average count per ml.	Classes according to standard plate count per ml.						
			10,000 or less		10,001 to 50,000		50,001 to 100,000		No.
			No.	No.	No.	No.	No.	No.	
No E.-A. organisms in 1 ml.	37	14,400	19	12	1	1	3	2	0
1.0 ml.	56	29,700	23	19	3	6	2	2	3
0.1 ml.	58	40,700	14	23	9	5	2	5	5
0.01 ml.	61	116,000	4	17	8	22	5	3	5
0.001 ml.*	58	892,000	0	3	7	16	3	3	29

\* Not necessarily limiting dilution.

isms, has undoubtedly been contaminated by those organisms subsequent to pasteurization. The presence of *Escherichia-Aerobacter* organisms in the finished product, whether because of faulty pasteurization or because of contamination following pasteurization, can thus be considered of sanitary significance.

*The phosphatase test:* The results of the phosphatase test are shown in table 4. The data emphasize the importance of running control tests on all samples of ice cream showing positive color reactions. One hundred and forty-three samples, or 45 per cent of the total number, showed some color development in the phosphatase test as usually made, and all of these samples would have been evaluated as under-pasteurized if control tests had not been run. Of this group, however, only 17 samples gave true positive tests in which the color development was definitely the result of enzyme activity. The color development observed in the remainder of the samples was caused by factors independent of the enzyme phosphatase.

TABLE 4  
*Phosphatase test results on 313\* commercial ice cream samples*

Relative amount of blue color	Number of samples	Percent of samples	Pasteurization evaluation	
			Number of samples classing as:	
			Satisfactory	Unsatisfactory
None	170	54.31	170	0
+	97	31.00	96	1
++	30	9.58	24	6
+++	16	5.11	6	10
Total	313	100.00	296	17

\* Test not run on 5 of the 318 samples.

Additional data on the seventeen samples positive to the phosphatase test, after suitable controls had been run, are presented in table 5. The standard plate count was above 100,000 per ml. on all but four of the 17 samples and was 1,900,000 or more per ml. in ten instances, indicating definitely unsatisfactory conditions as measured by the standard plate count. All of the phosphatase-positive samples contained *Escherichia-Aerobacter* organisms in 1 ml. quantities of ice cream, and these test organisms were found in 0.01 ml. or smaller quantities of ten of the samples. The extent to which under-pasteurization was responsible for the presence of the *Escherichia-Aerobacter* organisms in these samples is unknown. In view of the large number of samples which were negative to the phosphatase test and yet contained appreciable numbers of these test organisms, it would seem that contamination subsequent to pasteurization, as well as under-pasteurization, might have been an important factor contributing to their presence in considerable numbers as observed in this group of samples.

TABLE 5  
*Data on the 17 samples positive to the phosphatase test*

Sample No.	Phosphatase reaction	Standard plate count per ml.	Smallest quantity giving positive test for E.-A. organisms (ml.)	Total score minus bacterial score
B8 .....	+	17,000,000	0.0001	64
D12 .....	++	6,200,000	*0.001	61
4 .....	+	5,100,000	*0.000001	64.5
30 .....	+++	4,300,000	*0.01	63
C17 .....	++	2,300,000	0.1	64
C16 .....	+	2,200,000	*0.001	65
R34 .....	++	2,150,000	0.001	60
C32 .....	+	2,000,000	0.001	69.5
C11 .....	++	1,900,000	*0.001	64
42 .....	+	1,900,000	*0.01	67
BC11 .....	+++	480,000	0.01	61
R5 .....	+	380,000	0.1	61
D21 .....	+	130,000	1.0	65
BC29 .....	++	66,000	0.1	65
63 .....	++	60,000	1.0	66
BC27 .....	+	33,000	0.1	71
16 .....	+	19,000	0.1	64

\* The smallest quantity tested.

On the basis of total score minus bacterial score one phosphatase-positive sample scored 71 or more, one between 68 and 70.9, five between 64 and 69.9, six between 62 and 64.9, and four below 62. These data indicate that in this group of samples there was a marked tendency for a positive phosphatase test to be associated with undesirable characteristics of flavor, texture, body and color, particularly since only 36 samples in the whole survey scored between 62 and 64.9 and only 14 below 62.

Although there was a general tendency in this particular study for samples positive to the phosphatase test to represent poor quality ice cream, this relationship does not hold in all instances. The data indicate that ice cream may be satisfactory if judged on the basis of the other criteria used and yet be positive to the phosphatase test. The possible usefulness of the phosphatase test as a supplementary test in controlling the sanitary quality of ice cream is clearly indicated, both from the public health standpoint in checking pasteurization and because of the relationship which it may have to other quality characteristics of the product.

*Relation of butterfat test to score:* In table 6 the 318 samples of ice cream have been classified on the basis of butterfat test and total score minus bacterial score to determine whether a relationship exists between these two criteria of quality. Of the samples containing less than 10 per cent butterfat all except one scored below 68, but in each successively higher test class a large proportion of the samples was placed in the higher score classes, more than half of the samples containing 13.1 per cent or more of butterfat having scored 68 or above. In each successively lower score class a smaller

TABLE 6  
*The relationship between butterfat test and total score minus bacterial score*

Range of total score minus bacterial score	Samples in butterfat test range of:						Total samples				
	Below 10.0%		10.0-11.5%		11.6-13.0%						
	No.	% of score class	No.	% of score class	No.	% of score class					
71 or more (Excellent)	0	0	1	4.8	8	38.1	9	42.8	3	14.3	21
68.0-70.9 (Good)	1	1.0	11	11.3	45	46.3	36	37.1	4	4.1	97
65.0-67.9 (Fair)	16	10.7	46	30.6	57	38.0	29	19.3	2	1.3	150
62.0-64.9 (Poor)	6	16.7	10	27.8	12	33.3	7	19.4	1	2.8	36
Below 62 (Very poor)	3	21.4	5	35.7	4	28.6	2	14.3	0	0	14
Totals	26		73		126		83		10		318

percentage of the samples was found in the higher test classes and a larger percentage in the lower test classes.

Apparently a fairly definite relationship exists between butterfat content and total score minus bacterial score. This relationship may be due in part to the tendency for a manufacturer who is making a quality ice cream from the standpoint of high fat content to use good ingredients. Much of the effect undoubtedly is the result of the better flavor, body and texture associated with the use of larger amounts of butterfat in the mix. Only a small percentage of the manufacturers were marketing a product below the legal standard of 10 per cent for butterfat, and a definite tendency to use 12 per cent or more of butterfat in the ice cream was noted.

*Relation of weight per gallon to body and texture score:* In order to determine the relationship of weight per gallon to quality, 317 samples of ice cream were classified according to weight per gallon and body and texture score. This comparison is presented in table 7. Approximately one half of the samples weighed less than 4.5 pounds per gallon, which is equivalent to 100 per cent or more overrun. As the weight per gallon increased from less than 4 pounds per gallon to the 4.75 to 5.49 pounds per gallon range, the percentage of samples with body and texture scores in the upper brackets increased progressively. A marked tendency for greater percentages of the samples to fall in the lower score brackets was observed as the weight per gallon increased above 5.50 pounds. The five samples in the 5.50 to 6.49 pounds per gallon class which scored 24 or above for body and texture formed an exception to the general trend, an exception for which the explanation was not apparent.

A weight of 4.50 to 5.49 pounds per gallon seemed to be conducive to the best score, either unusually high or unusually low weights tending to be associated with undesirable body and texture characteristics. The value of 4.50 to 5.49 pounds per gallon corresponds to an overrun of from 66 to 102 per cent, a range which includes those values which are usually considered most desirable in good ice cream.

#### DISCUSSION

Each of the various tests and determinations used in this survey seemed to have its particular value for checking the quality of ice cream. This product is made from such a large number of ingredients and can be unsatisfactory because of such a variety of defects that a single determination, such as the standard plate count or the butterfat test, usually will show only the quality with respect to that particular factor and leave undetermined the quality with respect to other equally important factors. Although a rather close relationship existed between the evaluations placed on a sample by each of the several tests and determinations, complete characterization usually could be obtained only when several criteria were employed. An ice

TABLE 7  
*The relationship between weight per gallon and body and texture score*

Range in weight per gallon	Samples with body and texture score of:						Total samples
	24 or greater	23.0-23.9	22.0-22.9	21.0-21.9	20.0-20.9	% of wt. class	
No.	No.	No.	No.	No.	No.	% of wt. class	
Less than 4							
4.00-4.24	1	2.8	5	13.9	22	61.2	5
4.25-4.49	0	0	16	38.1	19	45.2	5
4.50-4.74	1	1.3	27	34.6	41	52.6	6
4.75-5.49	9	14.7	18	39.5	28	45.9	3
5.50-6.49	5	8.5	28	47.5	18	30.5	5
6.50-7.49	5	21.7	1	4.4	10	43.5	5
7.50-8.49	0	0	2	16.7	3	25.0	4
Totals	21	98	144	34	20	317*	

\* One sample not weighed.

cream might be satisfactory in all respects but one, weight per gallon or standard plate count, for example, and still be a poor ice cream because this one criterion indicated an undesirable condition with respect to raw materials or to processing.

In addition to the criteria now used in scoring ice cream, the weight per gallon, the number of *Escherichia-Aerobacter* organisms, the butterfat test, and the results of phosphatase test for adequacy of pasteurization might well be considered in the score card, as they give considerable added information concerning the quality of the product. Furthermore, each of these four criteria can be stated in absolute terms, placing more of the evaluation of a sample on a basis into which the variable factors of human judgment and taste do not enter.

#### SUMMARY AND CONCLUSIONS

1. The standard plate count, the minimum amount of sample containing *Escherichia-Aerobacter* organisms, the phosphatase test, the butterfat test, the weight per gallon, and the flavor, body and texture, color and package scores were determined on 318 samples of ice cream collected from over 300 Kansas ice cream manufacturers during July, 1938.
2. Standard plate counts of 100,000 or less per ml. were obtained on 59.8 per cent of all samples. A slightly larger percentage of the ice cream samples from counter-freezer operators than from wholesale manufacturers was in the lower count ranges.
3. Samples for which the standard plate count was 200,000 or less per ml. tended to have a high total score minus bacterial score, but when the count exceeded 200,000 per ml. no relationship between count and score was apparent.
4. A tendency for the standard plate count to increase as the smallest amount of ice cream containing *Escherichia-Aerobacter* organisms decreased was noted.
5. Only 17 of the 313 samples tested were positive to the phosphatase test. If suitable controls had not been run, 126 other samples would have been considered under-pasteurized or contaminated by unpasteurized dairy products.
6. The 17 samples positive to the phosphatase test were more or less unsatisfactory because of high standard plate count, large content of *Escherichia-Aerobacter* organisms, total score minus bacterial score or a combination of these three criteria.
7. Only 26 samples were found to contain less than 10 per cent butterfat. A tendency for samples of high butterfat content to have a high total score minus bacterial score was established.
8. A weight of 4.50 to 5.49 pounds per gallon was most frequently associated with high body and texture score; both lighter and heavier samples tended to be less desirable from this standpoint.

9. Despite certain relationships between the results obtained by use of the different determinations, there were enough instances in which these relationships did not hold to show that the use of a variety of tests and determinations is necessary in order to ascertain the true quality of a sample of ice cream.

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# INFLUENCE OF SOME MIX COMPONENTS UPON THE TEXTURE OF ICE CREAM

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## INTRODUCTION

Although ice-cream texture is most generally measured by the organoleptic test, some valuable information has been obtained by supplementing this test with other measurements. Thus Brainerd (1), Dahlberg (2), Cole (3), Reid (4), and others have presented data to show that ice-crystal size largely determines the texture of ice cream as judged organoleptically.

With this fact in mind we have used the dilatometer method for measuring the rate of freezing to see whether such data would indicate the ability of various ice-cream components to influence ice-cream texture (5). Some results obtained in 1933-1934 by one of us (6) giving microscopic measurements of the size of ice crystals formed during freezing are also included. Microscopic and dilatometer measurements were not made on the same sets of samples.

## METHODS

The three procedures used in this study were: 1, judging ice-cream texture organoleptically, 2, measuring ice-crystal size microscopically, and 3, measuring freezing rates of various samples by means of a dilatometer.

In the first case the scoring was done by at least two and usually three experienced judges who did not know the identity of the samples. For the microscopic observations the technique described by Cole (3) was employed. In the third case a dilatometer was used, as, previously described by this same author (8). The brine bath and the means of agitation and temperature control were essentially the same as those reported in the publication just mentioned.

Freezing in the dilatometer was accompanied by changes in volume, which in turn were indicated by differences in position of the mercury capillary in the apparatus. The size of samples for each set of comparisons was kept as uniform as possible, so that recording the time required for these differences to occur was considered a measure of the rate at which freezing took place.

Sweet cream and condensed skim milk or sweet butter and condensed skim milk were the sources of milk solids in the samples. The mixes were pasteurized at 150° F. and homogenized at pasteurization temperature using pressures from 1800 pounds per square inch to 2500 pounds per square inch, depending upon the fat contents of the mixes. The higher the fat content the lower the pressures used. In the mixes with high fat content, *e.g.*, 20 per

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cent fat and 2 per cent milk-solids-not-fat, it was necessary to run the mixes through the homogenizer two or three times in order to obtain satisfactory fat dispersion as judged by the microscopic appearance of the fat globules in the mix itself. The ice cream used for the organoleptic test and for the microscopic measurements was frozen in a multiple freezing unit consisting of four 1-gallon hand freezers rotating in a brine bath.

#### EXPERIMENTAL

The organoleptic test was used throughout the comparisons, and the results are given by comparing them with those of the other two methods. The dilatometer measurements will be considered first.

Preliminary observations with water and ice cream mixes in the dilatometer indicated the necessity of accurate temperature control during the freezing of samples. Lack of control caused significant variations in the rate of freezing. Obviously, then, to facilitate the satisfactory comparison of a series of samples their freezing points must be practically the same. This purpose was accomplished by adding suitable amounts of sodium chloride to the various samples in a given series so that all their freezing points were adjusted to that of the one with the lowest original value. The maximum range in freezing-point values for any series was  $0.05^{\circ}$  C. The degree to which the samples were supercooled before freezing also proved to be significant. In these comparisons each sample was supercooled  $2.50^{\circ}$  C.  $\pm 0.01$  and was held at this temperature four hours before freezing was initiated.

In addition, the rate of freezing varied considerably in all samples, depending upon the stage in the freezing process at which readings were obtained. It therefore became necessary to standardize on a procedure designed to secure representative values for the freezing rates in each case. These values were selected in the following manner.

The data for each sample were plotted on coordinate paper, using as ordinates (first) centimeters of expansion of the mercury capillary, and (second) expansion in centimeters was taken as a measure of the rate of freezing for each sample. This value was obtained by using that portion of the curve which most nearly represented a straight line—usually between 10 cm. and 20 cm. After tabulation of such data for the samples in a given comparison, they were plotted (figure 1).

The samples from which the data were obtained in this case were prepared so as to contain approximately 22 per cent milk solids, 15 per cent sugar, and 0.35 per cent gelatin (240 Bloom). Butter and condensed skim milk were used as the sources of milk solids. Although the proportion of milk fat to milk-solids-not-fat varied, the total per cent of milk solids remained approximately the same. According to the data, the time required for a given expansion to occur increased along with the proportion of milk-solids-not-fat.

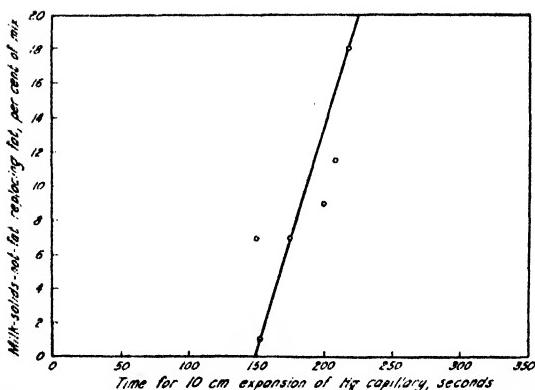


FIG. 1. Effect of replacing fat with milk-solids-not-fat upon rate of freezing (samples contained 0.35 per cent gelatin).

Another series of samples of essentially the same composition, except that cream instead of butter was the source of fat, yielded data that showed practically the same relationship.

TABLE 1

*Influence of milk fat and milk-solids-not-fat upon ice cream texture*

Sample	Composition		Texture placing	Remarks
	Fat per cent	Milk-solids-not-fat per cent		
1	20	2	6	Coarse
2	14	8	4	Slightly coarse
3	12	10	1	Smooth
4	10	12	2	Smooth
5	8	14	3	Slightly coarse
6	2	20	5	Slightly sandy

These samples all contained 15 per cent sucrose and 0.35 per cent gelatin.

Table 1 gives the results of scoring the samples in the first series mentioned. The values are the averages of the scores of two judges.

Since conceivably the gelatin content of the samples might mask the influence of the milk constituents, another series of samples was prepared, essentially the same in composition as the first two series mentioned except that no gelatin was included. The final data (figure 2) show the same relationship between the milk-solids-not-fat content and the retarding effect on freezing as that indicated in figure 1; but the correlation is somewhat better than in the preceding case, where the samples contained gelatin.

Unfortunately these samples were not judged organoleptically until three days after they had been frozen; and samples 5, 6, and 7 were sandy when scored. The results of the scoring are given in table 2.

As tables 1 and 2 indicate, the smoothest samples are not the ones with

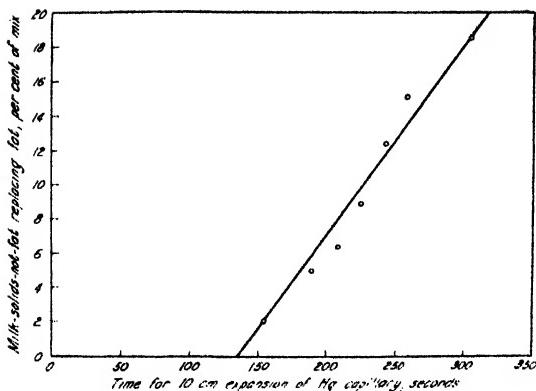


FIG. 2. Effect of replacing fat with milk-solids-not-fat upon rate of freezing (samples containing no gelatin).

the highest proportion of fat; rather, the best results (so far as texture was concerned) were obtained in the intermediate group. According to figures 1 and 2 the rate of freezing tends to be retarded as the proportion of milk-solids-not-fat increases.

TABLE 2  
*Influence of milk fat and milk-solids-not-fat upon ice cream texture*

Sample	Composition		Texture placing	Remarks
	Fat per cent	Milk-solids-not-fat per cent		
1	20	2	4	Coarse
2	16	6	3	Coarse
3	14	8	2	Slightly coarse
4	12	10	1	Smooth
5	8	14	5	Slightly sandy
6	6	16	6	Sandy
7	2	20	7	Very sandy

These samples all contained 15 per cent sucrose but no gelatin.

As already indicated, the microscopic measurements were made before the dilatometer observations were undertaken. Since they were carried out under similar conditions, however, and since the organoleptic test was used in both sets of experiments, it seemed advisable for comparative purposes to include those results which had direct application. The data on microscopic measurements are presented in tables 3, 4, and 5. All these samples were prepared with sweet cream and condensed skim milk as the sources of milk solids.

The results from two series of samples are given in table 3. The samples in a given series had the same percentages of fat but different percentages of milk-solids-not-fat. Thus samples 1, 2, and 3 all had 12 per cent fat but

TABLE 3

*Influence of increasing the percentage of milk-solids-not-fat in the mix upon the texture of ice cream*

Sample	Drawing temperature	Composition*		Average ice-crystal size <i>mm.</i>	Organoleptic test
		Fat	M.S.N.F.		
mm.					
1	- 5.6° C.	12	10.5	0.08 × 0.07	Coarse
2	- 6.5° C.	12	14.0	0.04 × 0.05	Smooth
3	- 6.8° C.	12	18.0	0.03 × 0.05	Very smooth
4	- 6.0° C.	22	3.1	0.05 × 0.07	Coarse-crumbly
5	- 6.5° C.	22	6.0	0.04 × 0.06	Smooth-good ice cream
6	- 6.0° C.	22	10.5	0.03 × 0.04	Smooth-gummy

\* All samples contained 15 per cent sucrose. Samples 1, 2, and 3 contained no gelatin. Samples 4, 5, and 6 contained 0.40 per cent gelatin (240 Bloom).

had milk-solids-not-fat contents varying from 10.5 per cent to 18 per cent. Samples 4, 5, and 6 all contained 22 per cent fat; and their milk-solids-not-fat content varied from 3.1 per cent to 10.5 per cent. In both sets, it will be noted, an increase in the milk-solids-not-fat was accompanied by a decrease in the average size of ice crystals with a simultaneous increase in smoothness of texture.

TABLE 4

*Influence of increasing the percentage of fat in the mix upon the texture of ice cream*

Sample	Drawing temperature	Composition*		Average ice-crystal size <i>mm.</i>	Organoleptic test
		Fat	M.S.N.F.		
mm.					
7	- 6.4° C.	22	10.5	0.03 × 0.04	Smooth-gummy
8	- 6.1° C.	18	10.5	0.04 × 0.05	Smooth
9	- 6.2° C.	12	10.5	0.06 × 0.05	Coarse
10	- 6.5° C.	22	6.0	0.04 × 0.06	Smooth-good ice cream
11	- 5.8° C.	18	6.0	0.04 × 0.07	Smooth-good ice cream
12	- 5.6° C.	12	6.0	0.06 × 0.07	Icy

\* All samples contained 15 per cent sucrose and 0.40 per cent gelatin (240 Bloom).

Table 4 gives the results from two sets of samples each having the same percentage of milk-solids-not-fat but having different percentages of fat in each sample. Samples 7, 8, and 9 contain 10.5 per cent milk-solids-not-fat, and their fat contents varied from 12 per cent to 22 per cent, whereas samples 10, 11, and 12 contained only 6 per cent milk-solids-not-fat but had fat contents corresponding to those just mentioned. As this table shows, an increase in the fat content in both cases resulted in a decrease in ice-crystal size and an increase in smoothness as determined organoleptically.

Table 5 presents results from three sets of samples, each having approximately the same percentage of total milk solids, but the individual samples varying in the proportions of fat and milk-solids-not-fat. One other variable

TABLE 5

*Influence of varying the proportion of fat and milk-solids-not-fat in the mix upon the texture of ice cream*

Sam- ple	Drawing tempera- ture	Fat	Composition*			Average ice- crystal size	Organoleptic test
			M.S.N.F.	Cond. skim and cream	Sodium casein- ate	Total milk solids	
13	- 6.5° C.	22	6.0	....	28.0	0.04 × 0.06	Smooth-good ice cream
14	- 6.1° C.	18	10.5	....	28.5	0.04 × 0.05	Smooth
15	- 6.5° C.	22	3.1	....	25.1	0.05 × 0.07	Course- crumbly
16	- 5.8° C.	18	6.0	....	24.0	0.04 × 0.07	Smooth-good ice cream
17	- 6.2° C.	12	10.5	....	22.5	0.06 × 0.05	Coarse
18	- 5.3° C.	12	7.5	3.0	22.5	0.04 × 0.04	**
19	- 4.6° C.	12	5.5	5.0	22.5	0.02 × 0.03	**

\* All samples contained 15 per cent sucrose and 0.40 per cent gelatin (240 Bloom).

\*\* Organoleptic tests were not available on these samples, as toluene was used to preserve the casein used in making the sodium caseinate.

was included, for samples 18 and 19 have three and five per cent respectively of the milk-solids-not-fat replaced with sodium caseinate. Judging from the results, as the proportion of milk-solids-not-fat increased in each set of samples the average size of the ice crystals decreased; and, furthermore, sodium caseinate is more effective in this respect than an equal amount of normal milk-solids-not-fat. Although the smooth-textured samples tend to have the smallest ice crystals, the evidence implies that there is at least one other factor contributing to smooth texture as judged organoleptically.

Photomicrographs were taken of practically all the samples examined microscopically, but it is not felt necessary to present them to emphasize the differences already pointed out in the tables.

Although not directly concerned with the subject at hand, it is an interesting fact that while examining samples of sandy ice cream with a polarizing microscope we were able to see very distinctly both lactose and ice crystals at the same time.

#### DISCUSSION

The evaluation of ice-cream texture is a complex problem, partly because the meaning of *texture* is not always clearly defined and partly because the organoleptic method generally used as a measure is undoubtedly influenced by more than one factor and is itself subject to the usual errors of human judgment.

If we take ice-crystal size and structure as the factors determining texture, then the microscopic method would seem to be a logical basis of evaluation.

On the other hand, if we consider the organoleptic test to be the best measure, we may suppose that our judgment of texture will be influenced by several contributing factors such as the size of the ice crystals, the temperature of the sample being judged, and the presence or absence of mix constituents that might act as lubricants. Since most of the results reported have been obtained by the latter method, we shall try to evaluate the factors contributing to the results reported.

With this in mind we can easily see why the results obtained by the three methods included in this study do not exactly coincide and also, to some extent, why various investigators have been led to conclusions that should possibly be modified.

The results obtained in this study and those reported by certain investigators lead us to conclude that if the organoleptic test is to be used as the criterion of smoothness or coarseness in ice cream, the lubricating effect of certain mix constituents, notably fat, as well as ice-crystal size, should be considered. In other words, one should not infer that a sample judged smoother than another sample necessarily has smaller ice crystals. Where the compositions of samples in a series are essentially the same, smoothness determined organoleptically would appear to be more nearly correlated with ice-crystal size than where the composition varies considerably, as in most of the comparisons made in this study.

If the results reported by Bradley and Dahle (7) are considered in this light, we might logically assume that the batch-frozen samples contained ice crystals large enough to be detected organoleptically unless there was sufficient fat or some other lubricant present to mask the coarseness due to these crystals. Hence high-fat ice creams would be more likely to taste smooth than lower-fat ice creams. With samples frozen in the continuous freezer, on the other hand, the ice crystals were probably so small that a smooth product resulted even though the fat content was low. In this case, therefore, one would not expect the differences in texture between high- and low-fat ice creams that would occur in samples frozen under less favorable conditions—for example, in batch freezers.

Here, apparently, is a likely reason for Dahlberg's (2) conclusion that fat was more effective than milk-solids-not-fat in producing smooth-textured ice cream. Judging from the results obtained in this study, the milk-solids-not-fat would retard the growth of ice crystals more than would fat; and consequently if size of ice crystals alone were considered as contributing to ice cream texture, milk-solids-not-fat rather than fat would be the more important. These findings can be justified on the following theoretical basis. Fat and water have little or no mutual affinity, hence the fat would not be expected to have much effect on the water phase. The proteins of milk, on the other hand, are hydrophylic and are therefore more likely to influence the water and its formation into ice crystals.

Be that as it may, the ultimate consumer will use the organoleptic test to determine texture along with other factors of quality. Our study agrees with Dahlberg's (already mentioned) and with many others in concluding that on this basis fat and milk-solids-not-fat are more effective in combination than they are alone in producing a smooth-textured product.

Our results are not conclusive proof of the point of view here expressed; but the theory is in keeping with them and is offered as one way of explaining our results as well as certain conclusions of other investigators.

#### SUMMARY AND CONCLUSIONS

1. The organoleptic test, the microscopic examination of ice-crystal size, and the dilatometer measurements of rate of freezing were used in this study as a basis for evaluating the factors contributing to ice-cream texture. The results obtained are briefly summarized below.

2. An increase in the percentage of milk solids as a result of increasing either the fat or the milk-solids-not-fat improved the smoothness of ice cream texture as judged organoleptically and tended to decrease the size of the ice crystals formed in ice cream.

3. When the milk solids content of the samples was maintained essentially the same but the ratio of fat to milk-solids-not-fat was varied significantly, the milk-solids-not-fat were more effective than the milk fat in causing the formation of small ice crystals.

4. Under these same conditions the dilatometer data show that the milk-solids-not-fat had a greater influence than did fat in retarding freezing. Hence it is suggested that the smaller ice crystals formed in samples with a higher proportion of milk-solids-not-fat may be due to a retarding action of the latter upon the growth of the ice crystals.

5. An increase in the proportion of fat in ice cream was found to have a greater effect upon texture judged organoleptically than it did in reducing ice-crystal size observed microscopically or in retarding the growth of ice crystals as measured by the dilatometer.

6. Where variations in composition are not too great, in a series of samples, one can expect good correlation between ice-crystal size measured microscopically and smoothness of ice cream judged organoleptically. This relation does not necessarily hold, however, if the proportion of fat to milk-solids-not-fat is very high or very low, possibly because fat in ice cream has a lubricating effect in the mouth, whereas this effect is not nearly so pronounced with milk-solids-not-fat.

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# CHANGES IN DIACETYL AND ACETYLMETHYLCARBINOL CONTENTS OF BUTTER AT VARIOUS TEMPERATURES<sup>1</sup>

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The work of various investigators has emphasized the importance of diacetyl as a flavor contributant of butter and other dairy products. The milk constituents in butter, especially the fat, have a rather characteristic flavor, but unless flavoring materials that commonly are produced by bacteria are also present the butter lacks the flavor desired by many consumers. The cultures ordinarily used to give butter a desirable flavor contain two general types of organisms; the citric acid fermenting type (*Streptococcus citrovorus* and *Streptococcus paracitrovorus*) produces a series of compounds, including diacetyl, from citric acid, while the lactic acid organism (*Streptococcus lactis*) establishes a pH that favors the accumulation of desirable fermentation products from citric acid. van Beynum and Pette (13) recently suggested a likely mechanism for the dissimilation of citric acid in which pyruvic acid is an intermediate.

Diacetyl is rather reactive toward many substances. Moreover, the citric acid fermenting streptococci which produce diacetyl under one set of conditions destroy it under other conditions, particularly at a favorable growth temperature and a relatively high pH. These general considerations led to a study of the changes in contents of diacetyl and its probable precursor, acetyl methylcarbinol, in butter made with different procedures and held at various temperatures.

## HISTORICAL

Following the discovery by van Niel, Kluyver and Derx (14) and by Schmalfuss and Barthmeyer (10, 11) that diacetyl and acetyl methylcarbinol occur in butter, the work of various investigators has substantiated the importance of diacetyl as a flavor constituent. Results of the earlier isolated analyses of butter for diacetyl have been critically reviewed by Barnicoat (1). The greater part of the analytical work on diacetyl and acetyl methylcarbinol thus far published deals with butter culture rather than with butter. Slatter and Hammer (12) studied changes in acetyl methylcarbinol plus diacetyl contents of butter held at different temperatures. Various workers, notably Barnicoat (1, 2), Mohr and Wellm (6) and Brioux and Jouis (4) determined changes in both diacetyl and acetyl methylcarbinol contents of butter by the

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use of different colorimetric methods; the methods have been reviewed (7, 9). Prill and Hammer (9) recently investigated changes in contents of these compounds during manufacture of butter.

#### METHODS

Except for one sample, all the butter studied was made in the butter laboratory of Iowa State College, largely in the general operation of the laboratory.<sup>3</sup>

The vats of cream, whether sweet or sour, contained approximately 30 per cent fat. Sour cream was neutralized to about 0.25 per cent acid prior to pasteurization; following pasteurization (150° F. for 30 minutes) and cooling, both sweet and sour cream were neutralized to about 0.10 per cent acid. The butter culture was made with addition of 0.15 per cent citric acid to the milk in order to increase the content of flavor contributants. Ordinarily, from 8 to 10 per cent of culture was added to cream and the mixture held cold for some hours (often overnight) before churning. The salt added was intended to give the butter a content of 2.25 per cent. Additional treatments or modifications in the manufacturing procedure are outlined under the experiments involved.

The butter was printed 1 or 2 days after manufacture, at which time the original analyses were made, and one-third lb. pieces were wrapped in parchment paper and placed at three different temperatures, namely, -10° to 0° F., 36° to 45° F. and 70° F. At suitable intervals samples were analyzed.

Diacetyl and acetyl methyl carbinol plus diacetyl were determined by the colorimetric method of Prill and Hammer (7). For the diacetyl determinations 100 gm. samples were used; for the acetyl methyl carbinol plus diacetyl determinations 20 gm. samples were distilled with ferric chloride added. Results are recorded as parts per million (p.p.m.).

#### RESULTS

Data on the changes in diacetyl and acetyl methyl carbinol plus diacetyl contents of butter at various temperatures are presented in four series of experiments. Each series involved butter made from the same original lots of cream by two procedures differing primarily with respect to only one factor.

##### *Series 1. Salted butter made with regular culture and with aerated culture*

The butter for each trial was made by dividing a vat of sweet cream and adding 10 per cent regular culture to one portion and 10 per cent aerated culture (3) to the other.

With aerated culture (table 1) both the diacetyl and the acetyl methyl-

<sup>3</sup> The churnings were made under the supervision of Dr. N. E. Fabricius, who is in charge of the butter laboratory.

TABLE I  
*Diacetyl (Ac<sub>2</sub>) and acetyl/methylcarbinol plus acetoin (Ac<sub>2</sub> + Ac<sub>3</sub>) contents of salted butter made with regular culture and with aerated culture and held at various temperatures. Butter made from sweet cream.*

Holding temp. of butter held	Trial 1				Trial 2				Trial 3				Trial 4			
	Culture used				Culture used				Culture used				Culture used			
	Regular	Aerated	Days butter held	Ac <sub>2</sub> p.p.m.	Regular	Aerated	Days butter held	Ac <sub>2</sub> p.p.m.	Regular	Aerated	Days butter held	Ac <sub>2</sub> p.p.m.	Regular	Aerated	Days butter held	Ac <sub>2</sub> p.p.m.
0° F.	0	.35	0.66	0	0.23	0.45	0	0.18	0.55	0	0.18	0.36	1.00	1.00	13.4	
-10° to 0° F.	96	.30	.66	.73	.23	.40	.64	.14	.55	.75	.17	.77				
	173	.31	.58	.150	.24	.35	.141	.11	.39	123	.17	.75				
				196	.24	.37	190	.13	.46	172		.33				
36° to 45° F.	8	.40	.67	.17	.24	.40	.10	.15	.61	11	.17	.86				
	42	.40	.64	.38	.23	.38	.29	.14	.49	21	.21	.88				
	61	.40	.56	.150	.17	.26	.39	.15	.45	45	.19	.86				
	95	.36	.60				101	.13	.31	123	.14	.25				
70° F.	4	.44	.73	5	.26	.45	.7	.15	.42	6	.19	.77				
	7	.45	.71	.16	.20	.40	.19	.17	.06	11	.20	.79				
	15	.44	.70	.29	.18	.39	.39	.09	.04	20	.18	.78				
	28	.35	.45	.58	.15	.26	.38	.08	.03	34	.14	.25				
	42	.31	.54	.47	.14	.13				45	.06	.23				

carbinol plus diacetyl contents of the butter (the carbinol plus diacetyl was determined only in trial 4) were conspicuously higher originally than when regular culture was used, as would be expected from analyses of the two types of culture (3, 9). This relationship persisted at the lower holding temperatures and in some cases at 70° F.

At -10° to 0° F. occasional slight decreases in diacetyl contents occurred. Presumably, these resulted from chemical changes not involving bacterial action. At 36° to 45° F. slight decreases in diacetyl contents were common but in a few instances, notably in trial 3 with aerated culture, there were conspicuous decreases. Even at 70° F. the diacetyl contents often were largely retained for rather extended periods, and this occurred when tallowy flavors and other defects developed in the butter. Some of the data suggest a slight increase in the diacetyl contents at the higher temperatures during the early part of the holding, particularly in trial 1 at 70° F. In general, with the high diacetyl contents obtained with the aerated culture the decreases in the contents were relatively greater than with the lower diacetyl contents obtained with regular culture.

*Series 2. Salted butter made with and without agitation  
of mixture of cream and culture*

In previous work (8) occasional agitation of a butter culture being held at a low temperature usually resulted in significant increases in diacetyl and the carbinol contents. These results suggested that introduction of oxygen through occasional agitation of a mixture of cream and culture during the holding would lead to an increase in diacetyl content of the mixture and of the butter made from it.

For each trial cream and 8 per cent culture were mixed in a small vat. Part of this mixture was drawn into cans and held cold while the remainder was stirred for 3 minutes every hour, the temperature being kept low. After 7 or 8 hours each portion was churned.

In trials 1, 2 and 3 (table 2) the agitation resulted in an increase in diacetyl and acetyl methyl carbinol plus diacetyl contents of the butter, but in trial 4, and several other trials not included, there were no appreciable differences. On holding the butter at the various temperatures there were, for the most part, only relatively slight changes in the diacetyl and the carbinol plus diacetyl contents.

*Series 3. Salted and unsalted butter made with culture*

In each of the two trials the same amount of the same culture was added to both sweet and sour cream. After partial working, a portion of the unsalted butter was removed while the remainder was salted and completed in the usual way. It should be noted that the unsalted butter was made from processed cream to which culture had been added but without the ripen-

TABLE 2  
*Diacetyl (Ac<sub>2</sub>) and acetyl methyl carbinal plus diacetyl (Ame + Ac<sub>2</sub>) contents of salted butter made with and without agitation of mixture of cream and culture and held at various temperatures. Butter made from sweet cream.*

Holding temp. of butter	Days butter held	Trial 1				Trial 2			
		Not agitated		Agitated		Not agitated		Agitated	
		Ac <sub>2</sub> p.p.m.	Ame + Ac <sub>2</sub> p.p.m.						
-10° to 0° F.	33 75	.09 .07	2.5 2.2	.17 .11	3.3 3.5	.40 68	.09 .07	.39 3.2	.14 .13
36° to 45° F.	4 11 20 33	.07 .08 .09 .10	3.0 2.3 2.4 2.6	.15 .14 .10 .10	3.9 3.3 3.1 2.7	8 15 25 49	.10 .11 .09 .08	.29 2.2 2.0 2.9	.14 .14 .15 .13
70° F.	3 11	.08 .12	2.9 2.5	.16 .13	4.1 3.6	4 8	.13 .12	.33 2.9	.18 .15
Trial 3									
Holding temp. of butter	Days butter held	Agitated				Not agitated			
		Ac <sub>2</sub> p.p.m.	Ame + Ac <sub>2</sub> p.p.m.						
		0	0.16	4.4	0.23	5.6	0	0.19	5.7
-10° to 0° F.	39 67	.13 .10	4.2 4.0	.19 .20	5.0 4.6	39 72	.18 .17	5.5 6.5	.17 .17
36° to 45° F.	8 14 24 48	.18 .16 .17 .16	4.8 4.0 4.8 4.7	.23 .23 .25 .20	5.0 5.2 5.4 4.8	10 14 25 53	.15 .20 .19 .20	6.5 6.3 5.9 6.4	.17 .19 .20 .19
70° F.	3 7	.19 .17	5.0 5.0	.26 .25	4.4 4.3	4 10	.21 .21	4.8 6.5	.18 .21

TABLE 3  
*Diacetyl ( $\Delta c_1$ ) and acetyl methyl glucarbinol plus diacetyl ( $Amc + \Delta c_2$ ) contents of salted and unsalted butter made with culture and held at various temperatures.*

Holding temp. of butter	Days butter held	Trial 1. Sweet cream				Trial 1. Sour cream			
		Per cent salt		0		Per cent salt		0	
		$\Delta c_1$ p.p.m.	$Amc + \Delta c_2$ p.p.m.	$\Delta c_2$ p.p.m.	$Amc + \Delta c_2$ p.p.m.	$\Delta c_1$ p.p.m.	$Amc + \Delta c_2$ p.p.m.	$\Delta c_2$ p.p.m.	$Amc + \Delta c_2$ p.p.m.
-10° to 0° F.	0	0.17	2.9	0.18	1.8	0.15	2.9	-	0.12
	33	.19	2.6	.19	2.2	.16	2.9	.13	2.2
36° to 45° F.	4	.20	2.5	.31	.6	.16	3.3	.18	2.8
	11	.25	2.7	.11	.7	.17	3.2	.19	2.6
45° F.	15	.20	2.7	.11	1.3	.16	3.1	.21	.4
	25	.20	3.2	.10	.6	.15	3.1	.26	1.4
70° F.	4	.22	3.0	.12	1.4	-	-	3.3	1.0
	8	.16	3.2	.12	2.7	.18	3.6	.08	1.3
Trial 2. Sweet cream									
Holding temp. of butter	Days butter held	Per cent salt				Per cent salt			
		2.25		0		2.25		0	
		$\Delta c_1$ p.p.m.	$Amc + \Delta c_2$ p.p.m.	$\Delta c_2$ p.p.m.	$Amc + \Delta c_2$ p.p.m.	$\Delta c_1$ p.p.m.	$Amc + \Delta c_2$ p.p.m.	$\Delta c_2$ p.p.m.	$Amc + \Delta c_2$ p.p.m.
-10° to 0° F.	0	0.14	4.9	0.17	5.6	0.20	5.8	0.21	5.7
	28	.13	4.4	.16	5.3	.21	4.7	.20	4.7
36° to 45° F.	70	.11	4.5	.12	4.6	.17	5.0	.18	4.2
	3	-	-	-	-	-	-	-	-
45° F.	6	.15	4.3	.25	5.9	.18	6.2	.24	5.6
	13	.16	5.5	.31	6.7	.17	5.5	.21	5.4
70° F.	20	.14	5.1	.42	11.2	.22	4.6	.19	4.0
	2	.14	5.5	.40	10.7	.20	5.9	.14	2.3
70° F.	4	.17	5.5	.75	33.6	.23	5.3	.60	30.5
	-	-	-	.73	25.4	.21	6.6	.40	14.3

TABLE 3—(*Continued*)

Holding temp. of butter	Special unsalted butter from highly ripened cream		
	Days butter held	Ac <sub>2</sub> p.p.m.	Amc + Ac <sub>2</sub> p.p.m.
	0	0.83	13.9
-10° to 0° F.	28	.70	13.5
36°	3	.59	17.0
to	9	.49	20.0
45° F.	16	.36	15.4

ing used when a high flavor is desired in unsalted butter; moreover, the working was less than normal although the butter was not leaky.

With the salted butter from both sweet and sour cream (table 3), changes in diacetyl and acetylmethylcarbinol plus diacetyl contents were slight. In the case of the unsalted butter made from sweet and sour cream the changes were slight at -10° to 0° F., but at temperatures permitting the activity of butter culture organisms very marked changes occurred. Apparently, the operation of unknown factors prevents generalizations in regard to the direction of these changes over a long period. Thus, in the unsalted sweet cream butter of trial 1 held at 36° to 45° F., the diacetyl values first increased and then decreased markedly, while the values for the carbinol plus diacetyl soon fell to low values; whereas at 70° F. the diacetyl contents tended to be lower than originally. In the unsalted sour cream butter of trial 1 held at 36° to 45° F., the diacetyl content increased progressively while the carbinol plus diacetyl content fell to low values; at 70° F. the content of these substances decreased. In the unsalted sweet cream butter of trial 2 held at 36° to 45° F. both diacetyl and carbinol plus diacetyl contents increased markedly, whereas at 70° F. the increases were still greater. In the unsalted sour cream butter of trial 2 held at 36° to 45° F. diacetyl and carbinol plus diacetyl contents decreased slightly, but at 70° F. there were marked increases followed by decreases.

In some of the unsalted samples acetylmethylcarbinol plus diacetyl contents decreased to such low values that the ratios of acetylmethylcarbinol plus diacetyl to diacetyl were much lower than ordinarily would be expected for butter made with culture.

The special sample of unsalted butter was obtained from a plant regularly making high flavored butter from processed sour cream ripened to a relatively high acidity. The original analyses were made as soon as the butter was received and show very high diacetyl and carbinol plus diacetyl contents. At 36° to 45° F. there were decreases in the diacetyl content and increases in the carbinol plus diacetyl content.

TABLE 4

*Diacetyl ( $Ac_2$ ) and acetyl methyl carbinol plus diacetyl ( $Ac_2 + Ac_2$ ) contents of salted butter made with culture and with added diacetyl and held at various temperatures. Butter made from sweet cream.*

Holding temp. of butter	Trial 1						Trial 2						
	Days butter held	Culture			Diacetyl			Days butter held	Culture			Diacetyl	
		$Ac_2$ p.p.m.	$Ac_2 + Ac_2$ p.p.m.	$Ac_2$ p.p.m.	$Ac_2 + Ac_2$ p.p.m.	$Ac_2$ p.p.m.	$Ac_2 + Ac_2$ p.p.m.		$Ac_2$ p.p.m.	$Ac_2 + Ac_2$ p.p.m.	$Ac_2$ p.p.m.	$Ac_2 + Ac_2$ p.p.m.	$Ac_2$ p.p.m.
-10° to 0° F.	0	0.25		4.4		0.51		0.6		0	0.22		4.4
	32	.25		4.1		.51				40	.23		5.4
65		.22		5.6						72	.23		5.2
													.26
36°	11	.26		5.6		.33		.9		9	.23		5.2
	19	.22		4.6		.40				20	.23		4.5
45° F.	46	.20		5.9		.41				37	.24		4.6
	68	.24		4.3		.35				51	.20		.27
70° F.	4	.29		5.0		.48		.9		4	.26		4.8
	11	.29		5.7		.46		.8		11	.26		5.4
Trial 3													Trial 4
Holding temp. of butter	Days butter held	Culture			Diacetyl			Days butter held	Culture			Diacetyl	
		$Ac_2$ p.p.m.	$Ac_2 + Ac_2$ p.p.m.	$Ac_2$ p.p.m.	$Ac_2 + Ac_2$ p.p.m.	$Ac_2$ p.p.m.	$Ac_2 + Ac_2$ p.p.m.		$Ac_2$ p.p.m.	$Ac_2 + Ac_2$ p.p.m.	$Ac_2$ p.p.m.	$Ac_2 + Ac_2$ p.p.m.	$Ac_2$ p.p.m.
	0	0.21		4.5		0.32		0.6		0	0.32		8.5
-10° to 0° F.	33	.19		4.4		.30				27	.28		7.2
	65	.19				.31				59	.27		7.7
36°	8	.24		4.7		.29				7	.33		7.9
	15	.25		5.0		.24				24	.30		.19
45° F.	44	.23		4.7		.24				48	.31		.15
	54	.24		4.8		.26				58	.31		.18
70° F.	4	.22		5.1		.24				3	.34		6.3
	8	.25		4.5		.22				7	.35		.19

*Series 4. Salted butter made with culture and with addition  
of diacetyl to the butter*

For each trial a batch of sweet cream was divided. To one portion 8 per cent culture was added, the mixture held overnight and then churned. The other portion was held overnight without culture added, and, after churning and washing, diacetyl, in the form of a dilute solution, was worked into the butter along with the salt. Although the amount of diacetyl added was calculated to make the concentration in the butter the same as the expected diacetyl content of the comparison butter, it was not possible to do this exactly.

In general, the changes in diacetyl and acetylmethylcarbinol plus diacetyl contents were relatively slight (table 4). In some cases the diacetyl contents of the butter made with added diacetyl decreased relatively somewhat more than did the diacetyl content of the butter made with culture, but these differences were never very marked. The acetylmethylcarbinol plus diacetyl contents of butter made without culture were somewhat greater than the content of added diacetyl. Presumably, this resulted from the carbinol present in the original cream which probably had undergone some slight bacterial action.

## DISCUSSION

The analyses of salted butter show a surprisingly high retention of diacetyl and of acetylmethylcarbinol, even at 70° F. The development of tallowiness at this temperature, which is common because of the great effect of relatively high temperatures on appearance of the defect, was not accompanied by a sharp decrease in the diacetyl or the carbinol content. Barnicoat (1, 2) also reported small changes in diacetyl contents of salted butter held frozen, at 14° to 17° F. or at 40° F. Reference should be made to the work of King (5) who showed that diacetyl promoted the oxidation of butter fat leading to tallowiness and loss of color with the concomitant destruction of the diacetyl. However, King used comparatively pure butter fat with a relatively high concentration of added diacetyl and, in some cases, rather drastic treatments, such as exposure to light. It is probable that in butter much of the diacetyl is in the water phase, rather than entirely in the fat as was the case in King's experiments. In general, it appears that the amount of diacetyl ordinarily encountered in butter has no significant effect in the promotion of chemical defects and that other factors, such as copper content of the butter and pH of the serum, are of more importance in this connection.

In unsalted butter significant changes in diacetyl and in acetylmethylcarbinol plus diacetyl occurred at 36° to 45° F. and at 70° F. and undoubtedly were due to activity of the butter culture organisms. The changes involved both increases and decreases, as would be expected from the general relationship of the butter culture organisms to these compounds. Slatter and Hammer (12) reported a striking production of acetylmethylcarbinol

plus diacetyl in unsalted butter at temperatures permitting activity of the butter culture organisms, and the results of Mohr and Wellm (6) also indicate significant changes in unsalted butter and lesser changes in lightly salted butter. An increase in diacetyl in unsalted butter is probably an important factor in the flavor development which this product often undergoes. A subsequent decrease presumably is accompanied by a partial loss of flavor.

#### SUMMARY

In general, salted butter (about 2.25 per cent salt) made with different manufacturing procedures showed only relatively slight changes in diacetyl and acetyl methyl carbinol plus diacetyl contents when held frozen or at 36° to 45° F. Even at 70° F., at which temperature chemical deterioration is especially rapid in butter, the contents of these substances were largely retained for considerable periods.

In the case of unsalted butter the changes at -10° to 0° F. were relatively slight, but at 36° to 45° F. or at 70° F. significant changes occurred, both increases and decreases being involved.

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## EFFECT OF SALTING CURD FOR BLUE CHEESE<sup>1</sup>

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The salt used in various dairy products has a number of effects. It definitely influences the flavor, and even with such products as milk, buttermilk and ice cream it may be employed to bring out flavor. In the concentrations used with certain products, salt has pronounced preserving powers. For example, over a wide temperature range salted butter ordinarily undergoes microbiological changes more slowly than unsalted butter. Organisms differ in their salt tolerance, and as a result a certain concentration may greatly modify the flora of a product by largely preventing development of certain species and having little effect on others.

In the manufacture of blue cheese, salt is incorporated by covering the surface with dry salt or by floating the cheese in brine. Salting is usually begun when the hoops are removed the day following preparation of the curd. Certain blue-veined cheeses commonly have a relatively high salt content and such a content, together with one or more additional factors, favors the dominance of the characteristic mold in the cheeses (1, 3).

Preliminary studies suggested that in the manufacture of blue cheese there were certain advantages in adding some salt to the curd at the time of hooping, and accordingly more detailed studies were carried out.

### GENERAL PROCEDURE

The effect of salting curd for blue cheese was studied with cheese manufactured from homogenized milk (2). In making each comparison, the curd necessary for two cheeses was dipped on a cloth, and as soon as draining was reasonably complete, mold powder was mixed with the curd. One hoop was filled with half the curd; salt (2 per cent in most trials) was mixed with the remainder after which it was put into a hoop. The cheeses were then finished in the usual manner and salted with dry salt. At intervals during a 4-month ripening period the cheeses were examined by cutting segments from them; after each examination the freshly cut surfaces were paraffined.

### RESULTS

In the early trials the amounts of salt mixed with the curd varied from 2 to 5 per cent of the estimated curd weight. Addition of more than 2 per cent salt tended to destroy the fusing properties of the curd particles and to retard development of mold so the later comparisons were made entirely with 2 per cent.

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Most of the trials were carried out in connection with a study of the effect of various strains of mold on ripening of blue cheese, in which mold powders prepared from the strains were mixed with portions of the same curd. Thus, a series of comparisons involved the use of various molds, employed with and without salting of the curd, on the same original lot of curd.

TABLE 1

*Mold growth, color and flavor of blue cheese made with and without salting of curd*

Series no.	Compari- son no.	Mold growth		Color	Flavor	
		salted	unsalted		salted	unsalted
9	1	good	good	salted lighter	fair	butyric*
	2	good	good	no difference	good	good
	3	good	fair	salted lighter	fair	musty
	4	good	poor	salted lighter	good	fermented
	5	good	good	no difference	good	good
	6	good	good	salted lighter	fair	butyric
	7	good	poor	salted lighter	good	fair
	8	good	good	salted lighter	good	fair
	9	good	poor	salted lighter	fair	butyric
	10	good	good	no difference	fair	fair
	11	good	good	salted lighter	good	fair
	12	good	fair	salted lighter	good	good
10	1	good	good	salted lighter	good	good
	2	good	good	salted lighter	good	good
	3	good	good	salted lighter	good	fair
	4	good	good	salted lighter	fair	fair
	5	good	poor	salted lighter	fair	butyric
	6	good	fair	salted lighter	musty	musty
	7	good	good	salted lighter	good	good
	8	good	good	salted lighter	good	butyric
	9	good	poor	salted lighter	fair	fair
	10	good	good	salted lighter	musty	musty
	11	good	fair	salted lighter	fair	musty
	12	good	good	salted lighter	good	fair
	13	poor	poor	salted lighter	unclean	unclean

\* Flavor suggests butyric acid.

The mold growth, color and flavor of two representative series of cheese, made with and without salting the curd, are given in detail in table 1. The data show that salting the curd had an effect on mold development in the cheese. With 5 of 12 comparisons in series 9, and with 4 of 13 comparisons in series 10, mold growth was considered more satisfactory when the curd was salted than when it was not; in the remaining comparisons no appreciable difference was noted. Cheese from salted curd was lighter in color, and therefore was more desirable from the color standpoint, than cheese from unsalted curd with 9 to 12 comparisons in series 9 and with all comparisons in series 10. The flavor of cheese from salted curd tended to be superior to that of cheese from unsalted curd. Certain flavors, such as musty, fermented or butyric, were sometimes present in cheese from unsalted curd when they were absent in cheese from salted curd, but salting the curd did not entirely eliminate off flavors.

Table 2 summarizes results obtained in 10 series of cheese made with and without salting the curd. The effect of the salting was essentially the same

TABLE 2

*Summary of mold growth, color and flavor of blue cheese made with and without salting of curd*

Series no.	No. comparisons	Mold growth		Color		Flavor*			no difference
		salted better	no difference	salted lighter	no difference	salted better	unsalted better		
1	8	3	5	6	2	4	1	3	
2	8	5	3	8	0	3	0	5	
3	8	4	4	6	2	5	0	3	
4	8	1	7	5	3	4	1	3	
5	8	3	5	8	0	6	0	2	
6	8	5	3	8	0	3	1	4	
7	8	4	4	7	1	6	0	2	
8	8	3	5	8	0	4	1	3	
9	12	5	7	9	3	8	2	2	
10	13	4	9	13	0	6	1	6	

\* In some cases when both cheese in a comparison were given the same general flavor description, one was better than the other; such differences are taken into account in the tabulation.

as in the individual series, in that mold growth was sometimes improved, the color was rather regularly lighter and there was less tendency to develop off flavors.

#### DISCUSSION

When salt is mixed with blue cheese curd just before hooping, a portion of it is carried away in the whey that drains from the curd. Presumably, the percentage lost is not constant. However, with normal salting of the cheese, no serious irregularities in salt content of the ripened product were encountered.

The somewhat better mold growth in the cheese when the curd was salted probably was due primarily to the comparatively open texture that accompanied this procedure. The lighter color that rather regularly resulted presumably was due to an increase in stability of the curd, which kept the fat well dispersed; in studies on delayed salting of blue cheese, Lane and Hammer (2) noted that the more the salting was delayed the deeper became the color. The effect on the flavor suggests that the salt in some cases may have limited the growth of certain undesirable organisms. Since off flavors sometimes were encountered with salting of the curd, the inhibition was by no means complete.

By salting the curd, the general effects of salt on the cheese are initiated sooner than without such salting because the salt penetrates from the surface rather slowly.

In blue cheese manufacture, salting of the curd has become standard

practice in the cheese laboratory at Iowa State College. It also has been found useful in certain commercial plants.

#### SUMMARY

In the making of blue cheese, addition of 2 per cent salt to the curd just before hooping sometimes resulted in better mold growth in the cheese, rather regularly gave a lighter color and tended to control certain flavor defects.

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# RELATIONSHIP OF ACID NUMBER VARIATIONS TO THE QUALITIES AND FLAVOR DEFECTS OF COMMERCIAL BUTTER<sup>1</sup>

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Changes occurring in the fat of butter are of great importance from the standpoints of flavor and keeping quality of the product. Hydrolysis of the fat may set free some of the lower fatty acids, particularly butyric, caproic and caprylic, causing a condition commonly referred to as rancidity, constituting one of the most serious defects occurring in butter. Rancidity frequently develops in samples of commercial butter when they are subjected to keeping quality tests.

In butter made from unheated cream the lipase normally present in milk may cause fat hydrolysis. However, this enzyme is readily destroyed by the usual pasteurization procedure. Since there is little opportunity for significant recontamination of pasteurized cream or of butter with lipase, it probably has little effect on the keeping qualities of commercial butter.

Many micro-organisms are able to hydrolyze butterfat. Organisms of this type are widespread in nature, often being present in raw cream, water and dairy plant equipment. They are ordinarily killed by pasteurization but recontamination after pasteurization may occur. If such organisms gain entrance to pasteurized cream in sufficient numbers and find conditions suitable for growth, they may cause serious defects in the resulting butter. Salt retards the growth of these organisms, so that they produce the most serious defects in unsalted butter.

The acid number has proved to be a valuable adjunct to the organoleptic method of determining the degree of hydrolysis in the fat of cream or butter. However, certain organisms may utilize fatty acids as food. Also, when the cream acidity is standardized the acids in the fat as well as those in the serum are partially neutralized. Under such conditions the acid number is not an exact index to the degree of fat hydrolysis.

Samples of commercial butter of varying qualities were studied to determine any possible correlation between acid number of the fat and quality of the butter. Both unsalted and salted butter were used.

## ACID NUMBER OF BUTTERFAT

The usual method of expressing the acid number of fat is as the number of milliliters of N/1 alkali required to neutralize the free acid in 100 gm. of fat. All references to acid number will imply this meaning. The butter

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samples were melted, the fat aspirated off and filtered through paper. The samples were melted and filtered in a 45° C. incubator.

The acidity of the fat was determined by the method devised by Breazeale and Bird (1). Ten gm. of filtered fat were weighed into a 125 ml. Erlenmeyer flask, 25 ml. of petrolic ether and 10 ml. of absolute ethyl alcohol were added, and the contents of the flask thoroughly mixed. The petrolic ether dissolved the fat and fatty acids and the alcohol dissolved any soap formed during the titration. Ten drops of alcoholic phenolphthalein were added and the sample was titrated against N/10 potassium hydroxide made up in absolute alcohol. The number of milliliters of N/10 potassium hydroxide required to neutralize the free acid in 10 gm. of sample corresponded to the acid number of the fat.

#### UN SALTED BUTTER

The unsalted butter came from various Iowa creameries and was obtained through a marketing association. The samples were examined for flavor defects immediately on receipt and after 2, 4 and 6 days at 21° C. After the 6 days, the fat acid numbers were determined. Whenever sufficient quantities of samples were available, acid numbers of the fat of the fresh butter were also determined.

Table 1 shows the data on 14 samples of butter. The samples were divided into two groups, those not developing rancidity during holding at 21° C. and those which did. The acid numbers of the fat were not determined in the

TABLE 1  
*Relationship of acid number to the general quality of unsalted butter (Samples from various Iowa creameries)*

Sample	Flavor of butter after incubating at 21° C.			Acid number of fat after 6 days at 21° C.
	2 days	4 days	6 days	
Samples not developing rancidity				
1 .....	good	good	good	3.4
2 .....	good	good	good	2.6
3 .....	good	good	good	3.6
4 .....	good	good	good	4.0
5 .....	good	good	good	13.6
6 .....	good	good	good	2.3
7 .....	good	good	good	1.0
8 .....	good	good	good	2.9
9 .....	good	good	good	2.4
Samples developing rancidity				
10 .....	skunk odor	skunk odor	rancid	4.8
11 .....	ester odor*	rancid	v. rancid	6.2
12 .....	ester odor*	rancid	sl. rancid	10.8
13 .....	ester odor*	rancid	rancid	5.6
14 .....	rancid	rancid	rancid	5.8

\* The ester odor definitely suggested the odor produced in butter by *Ps. fragi*.

fresh samples but judging from titrations of many similar samples, it is probable that the original acid numbers were all less than 1.0. In examination of these samples particular attention was given to the detection of rancidity.

In general, the samples which became rancid developed the higher acid numbers. Samples 10 to 14 inclusive became rancid and in general the acid numbers were considerably higher than of the non-rancid samples. Sample 5 was an exception having a good flavor and yet having an acid number of 13.6; also samples 1, 3, 4 and 8 had higher acid numbers than are usually found in non-rancid butter.

The results on another series of 32 samples are given in table 2. The samples were again divided into those not developing rancidity and those

TABLE 2

*Relationship of acid number to the general quality of unsalted butter (Samples from various Iowa creameries)*

Sample	Flavor of butter after incubating at 21° C.			Acid number of fat	
	2 days	4 days	6 days	Fresh	After 6 days at 21° C.
Samples not developing rancidity					
1	cheesy	cheesy	cheesy	0.7	1.2
2	good	good	good	.7	.8
3	good	good	good	.7	.9
4	good	good	good	.7	.8
5	good	good	good	.6	13.6
6	good	good	good	.7	2.8
7	good	good	good	.6	1.8
8	good	good	good	.6	.9
9	good	good	good	.9	1.3
10	good	good	good	.6	1.1
11	good	good	good	.5	1.0
12	good	good	good	.7	1.8
13	good	good	good	.8	2.2
14	good	good	good	.7	1.6
15	good	good	good	.7	3.1
16	good	good	good	.7	9.8
17	good	good	good	.7	1.0
18	good	good	good	.6	1.0
19	good	good	good	.6	1.4
20	good	good	good	.7	1.0
21	cheesy	cheesy	cheesy	.7	1.5
22	good	good	good	.8	1.8
23	good	good	good	.7	1.4
24	good	good	good	.7	1.0
25	good	good	good	.6	11.6
Samples developing rancidity					
26	good	good	sl. rancid	.7	2.8
27	good	good	sl. rancid	.6	3.2
28	sl. rancid	rancid	rancid	1.1	2.4
29	good	good	sl. rancid	.7	14.0
30	cheesy	sl. rancid	sl. rancid	.8	4.8
31	sl. rancid	rancid	rancid	.8	7.6
32	sl. rancid	sl. rancid	sl. rancid	.6	5.2

which did. The acid numbers of the fat when the butter was received were below 1.0 in all cases except sample 28, which was 1.1. After 6 days at 21° C., the acid numbers of the non-rancid samples ranged from 0.8 to 11.6; the rancid samples from 2.4 to 14.0. In general, the non-rancid samples had relatively low fat acid numbers, 2, 3, 4, 8, 9, 10, 11, 17, 18, 20 and 24 having acid numbers of 1.0 or slightly higher after 6 days at 21° C. Samples 16 and 25 were exceptions with acid numbers of 9.8 and 11.6 respectively. Of unusual interest were samples 28 and 31. Neither sample was rancid when received but within 2 days sample 28 became *slightly* rancid and only increased from the original acid number of 1.1 to 1.6. Sample 31 became *distinctly* rancid during the same period and only increased from 0.8 to 1.3.

It may be noted in the samples developing rancidity after holding, that other flavor defects frequently preceded the rancid odor and flavor. In some cases an ester odor was the first indication of the approach of rancidity. The ester odor definitely suggested the odor produced in butter by *Ps. fragi*. In every sample in which the ester odor was present, rancidity soon followed. A cheesy flavor occasionally preceded rancidity; however, all samples showing cheesy flavor did not become rancid during the 6 day holding period.

These contrasting conditions in which some of the non-rancid samples had high acid numbers and some rancid samples had low acid numbers agree with the findings of Guthrie (2) that there is little correlation between the acid number of butterfat and the development of rancidity. It is possible that some lipolytic organisms have a selective action on certain of the glycerides of the fatty acids. In the one case only the higher acids may be liberated which yield increased acid numbers on the fat without causing serious off-flavors. Other organisms may liberate primarily the lower fatty acids including a small quantity of butyric acid, which while insufficient to increase the acid number, may cause a rancid flavor. Results reported later show that certain organisms, particularly *O. lactis*, when growing in cream or butter liberate only a very small amount of volatile acid from the fat. This may be a selective action on the fats or the lower acids may be consumed by the growing cells as rapidly as they are liberated as suggested by Orla-Jensen (3).

TABLE 3

*Relationship of acid number to the general quality of salted butter (Samples entered in the 1938 National Cold Storage Contest)*

Sample	Score	Acid number
1 .....	94	0.6
2 .....	92	.6
3 .....	92	.5
4 .....	92	.6
5 .....	95	.8
6 .....	94	.6
7 .....	93	.6
8 .....	94	.7

## SALTED BUTTER

The salted butter came from various scoring contests and exhibits and in general was two or more weeks old when received.

The data in table 3 show the acid numbers of 8 samples of fine quality, lightly salted contest butter scoring 92 to 95. These samples were obtained from creameries submitting entries in the 1938 National Cold Storage butter contest. The acid numbers of the samples ranged from 0.5 to 0.8 and were little different from the acid numbers of fat of average quality commercial salted butter as shown in table 4.

Table 4 shows the acid numbers of the fat of 27 samples of butter from an Iowa State College Educational Butter Scoring Contest. Some of these

TABLE 4

*Relationship of acid number to the general quality of salted butter  
(Samples from Iowa State College Educational Butter Scoring Contest—some made from neutralized cream)*

Sample	Origin of butter	Score	Acid number
1	Iowa	93.0	.8
2	Iowa	92.5	.7
3	Texas	90.0	.6
4	Iowa	91.5	.7
5	Iowa	92.0	.5
6	Iowa	91.5	.8
7	Iowa	92.0	.7
8	Oregon	91.0	.8
9	Nebraska	91.5	.7
10	Iowa	91.5	.7
11	Iowa	92.0	.6
12	Iowa	93.0	.7
13	Iowa	90.0	.7
14	Iowa	90.5	.7
15	Iowa	91.0	.5
16	Iowa	91.0	.7
17	Iowa	91.0	.5
18	Iowa	91.0	.8
19	Iowa	91.5	.7
20	Iowa	91.5	.6
21	Iowa	90.5	.5
22	Iowa	91.0	.7
23	Iowa	91.0	.7
24	Iowa	91.0	.6
25	Iowa	90.0	.6
26	Iowa	90.5	.6
27	Iowa	90.5	.7

samples were made from neutralized cream. The scores ranged from 90 to 93 and the acid values from 0.5 to 0.8. Judging from the scores of the lots of butter there must have been considerable difference in the qualities of the cream from which they were made. It appears that there was little correlation between acid numbers of the fat of neutralized cream butter and the quality of the cream from which it was made. In fact sample 1 a 93 score butter, had an acid number of 0.8 while sample 3, a 90 score butter, had an acid number of 0.6.

The data shown in table 5 indicate that Oklahoma butter exhibited at the Oklahoma State Fair had slightly higher fat acid numbers than Iowa

TABLE 5

*Relationship of acid number to the general quality of salted butter  
(Samples exhibited at the 1938 Oklahoma State Fair)*

Sample	Score	Acid number
1 .....	92.0	1.4
2 .....	93.0	.7
3 .....	92.5	.6
4 .....	93.0	.8
5 .....	90.5	.5
6 .....	92.0	.8
7 .....	92.0	1.0
8 .....	89.0	2.2
9 .....	93.0	.6
10 .....	92.0	.6
11 .....	92.0	.6
12 .....	89.0	1.8
13 .....	89.5	.9
14 .....	92.5	.6
15 .....	90.0	.7
16 .....	93.0	.5
17 .....	90.0	.8
18 .....	89.5	.5
19 .....	88.5	1.2
20 .....	91.5	1.7
21 .....	90.0	.9
22 .....	90.0	1.0
23 .....	90.0	1.0
24 .....	90.5	.9
25 .....	91.0	.9

butter of similar quality. The exact age of these samples and the conditions under which they were manufactured were not known. Possibly the age and quality of the cream when churned, age of the butter and period of lactation of the producing cows may have influenced the acid values.

#### DISCUSSION

In reviewing the studies on unsalted and salted commercial butter of widely varying qualities, certain observations should be pointed out. There was no definite relationship between the acid number of the fat of unsalted butter and flavor defects. The fat of fresh unsalted butter invariably had low acid numbers and increases after holding 6 days at 21° C. were always evident. About 25 per cent of the samples of unsalted commercial butter observed became rancid during the holding period. In the samples not developing rancidity with a few exceptions, the increases in the acid numbers were small. In the samples developing rancidity however, the increases were significant although exceptions to this generalization were also encountered. Some samples of unsalted butter of good flavor had very high acid numbers after the holding period while certain rancid samples had low acid numbers.

Many samples of salted butter were subjected to keeping quality tests but since very few of the samples became rancid, acid values were not determined after the holding period. The acid numbers of the fat of fine quality, lightly salted contest butter were similar to those of commercial salted butter of considerably lower quality. There was no correlation between the scores of salted butter and the acid numbers of the fat.

#### CONCLUSIONS

1. Most samples of unsalted butter increased in acid numbers of the fat during holding for 6 days at 21° C.
2. When samples of commercial unsalted butter were held at 21° C., approximately 25 per cent became rancid within 6 days.
3. No close correlation existed between the acid number of the fat and the quality of commercial unsalted butter; butter of good quality often had relatively high acid numbers, while some rancid samples had relatively low acid numbers.
4. When samples of commercial salted butter were held at 21° C., comparatively few of the samples became rancid in 6 days.

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# THE COMPARATIVE NUTRITIVE VALUE OF BUTTER FAT AND CERTAIN VEGETABLE OILS\*

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In our early work on the relation of fat to the utilization of lactose in milk (1) it was noticed that when feeding different fats as supplements to skimmed milk diets the young rats grew slightly faster and appeared to have a better coat of hair when fed butter fat as compared with corn oil or coconut oil. Because of these observations it was thought advisable to carry out specific feeding trials with different fats incorporated into a skimmed milk diet.

The work of Evans and Burr (2), Burr and Burr (3), and Evans and Lepkovsky (4) has established the fact that certain unsaturated fatty acids are necessary in the nutrition of the rat. These workers have shown that rats placed on fat-low diets develop a deficiency disease which is corrected by incorporating small amounts of linoleic, linolenic, or arachidonic acids in the diet. Holt (5) has reported that olein, olive oil, and soybean oil are superior to butter fat in the nutrition of premature infants because these fats are more readily absorbed than butter fat. Recently Gullickson and Fountaine (6) have observed the superior nutritive value of butter fat over certain vegetable oils in the nutrition of the calf. Aside from these studies we are unaware of any clear-cut evidence that one fat is superior to another in normal nutrition.

## EXPERIMENTAL

Weanling rats about 21 days old and weighing about 40 grams or less were used for these studies. The different fats were separately homogenized into skimmed milk with a small hand homogenizer. A small portion of skimmed milk was heated to about 40° C. and the fat to be added was weighed out, melted, and the two ingredients homogenized. The homogenized material was then diluted with cold skimmed milk to bring the fat content to 4 per cent. Twenty micrograms of crystalline  $\beta$ -carotene were added to each gram of fat except butter fat to which were added ten micrograms of  $\beta$ -carotene as a source of vitamin A. All animals were irradiated ten minutes each day. The fats used were fresh and the butter fat was obtained from salt-free butter. Fresh skimmed milk, obtained daily from the University Creamery, was used throughout the ex-

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periments. Analysis showed that this skimmed milk contained 0.04 per cent of butter fat. All milks were mineralized with iron, copper, and manganese so that each 100 cc. of milk contained 1.5 mg. of iron, 0.15 mg. of copper, and 0.15 mg. of manganese.

For each experiment three males and three females were placed on each milk and all animals were kept in separate cages. The animals were treated as nearly alike as possible and were fed the milk *ad libitum* but care was taken not to give an animal much more than it would consume in a day because of the danger of fat separation and the animal consuming more fat in proportion to other ingredients in the milk. All animals were weighed weekly.

In the first trials the following diets were fed:

Skimmed milk plus butter fat	to make four per cent fat
" " "	corn oil " " " " "
" " "	coconut oil " " " " "

In later trials cottonseed oil and soybean oil were fed in the same manner as the above fats. The results from the first experiments indicated that the rats on butter fat made better gains and were much better appearing during the first three weeks of the experiment than the animals on corn oil or coconut oil. These animals were carried through to maturity and the females in each group mated with a male from the same group. All females on the three fats showed normal estrous cycles. Normal litters were obtained from all females on butter fat and all pups were raised to maturity. The litters from the females on corn oil were usually small and some of the pups were weak and died shortly after birth. The mother would usually eat the rest of the litter within a few days. Occasionally the udders of the females on corn oil would be caked, swollen, and sensitive to touch. No litters were obtained from the females on coconut oil. However, the females became pregnant but the fetuses were reabsorbed, which suggested a vitamin E deficiency. Administration of 2-3 mg. of pure natural  $\alpha$ -tocopherol relieved the reproduction difficulties, but the pups were in very poor condition. No pups obtained from the females on corn oil or coconut oil after administration of  $\alpha$ -tocopherol could be raised and the experiment had to be discontinued. In subsequent experiments 100 micrograms of synthetic  $\alpha$ -tocopherol, dissolved in alcohol, were added to all milks once each week. No minerals were added to the milk on the days when the  $\alpha$ -tocopherol was fed in the milk.

The results obtained on several successive experiments representing 36 rats on each fat supported the earlier observations that the rats on butter fat made more rapid gains during the first three weeks of the experiment than the rats on corn oil and coconut oil. In recent experiments 12 rats on cottonseed oil and 6 rats on soybean oil were included in the feeding experiments. The growth obtained on the latter fats incorporated into skimmed

milk was similar to that obtained on corn oil and coconut oil during the first two or three weeks. After this period of time the animals on the vegetable oils grew as well as the animals on butter fat but still were inferior in appearance; the hair coat being coarser and dull in appearance. The weight records of these animals are represented in charts I and II. The curves in

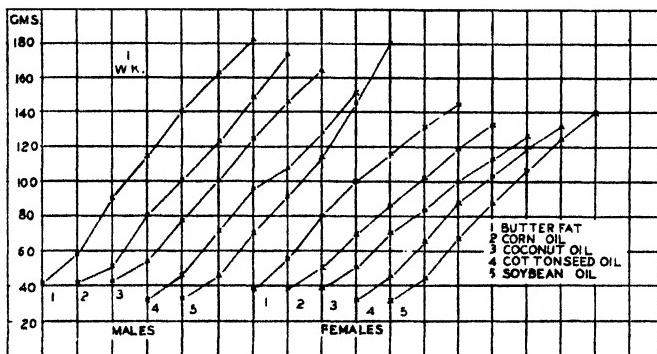


CHART I. Curves showing average weights for each week for male and female rats, representing 36 rats (18 males, 18 females) on each of the following fats: butter fat, corn oil, and coconut oil, 12 rats (6 males, 6 females) on cottonseed oil, and 6 rats (3 males, 3 females) on soybean oil.

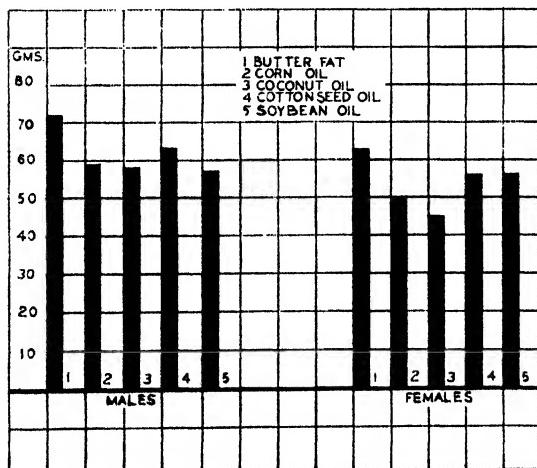


CHART II. Average gain made during the first three weeks on experiment by male and female rats representing 36 rats (18 males, 18 females) on each of the following fats, butter fat, corn oil, and coconut oil, 12 rats (6 males, 6 females) on cottonseed oil, and 6 rats (3 males, 3 females) on soybean oil.

chart I represent the average weights for each week for the male and female rats. Chart II represents the gain made by the male and female rats during

the first three weeks on all experiments. The gains made by the male rats on butter fat during the first three weeks were 22 per cent greater than on corn oil, 23 per cent greater than on coconut oil, 14 per cent greater than on cottonseed oil, and 26 per cent greater than on soybean oil. The gains made by the female rats on butter fat on the same experiment were 24 per cent greater than on corn oil, 38 per cent greater than on coconut oil, 9 per cent greater than on cottonseed oil, and 9 per cent greater than on soybean oil.

In view of the fact that butter fat consistently gave a better growth response, it was considered advisable to add the non-saponifiable fraction of butter fat to corn oil and coconut oil in order to make a more comparable experiment and determine if the growth differences still existed. The non-saponifiable fraction of butter fat was prepared as follows (7). One hundred grams of melted butter fat were poured into 200 cc. of 20 per cent alcoholic potassium hydroxide solution and the saponification allowed to proceed for four hours at 37° C. The material was then diluted with 800 cc. of water and extracted several times with ether. The ether solution was washed twice with water and evaporated to dryness under vacuum. The remaining material was then taken up in 100 grams of the oil to be fed. Since ten micrograms of  $\beta$ -carotene were added to each gram of butter fat used for making the butter fat milks, ten micrograms of carotene were added to each gram of corn oil and coconut oil containing the non-saponifiable ma-

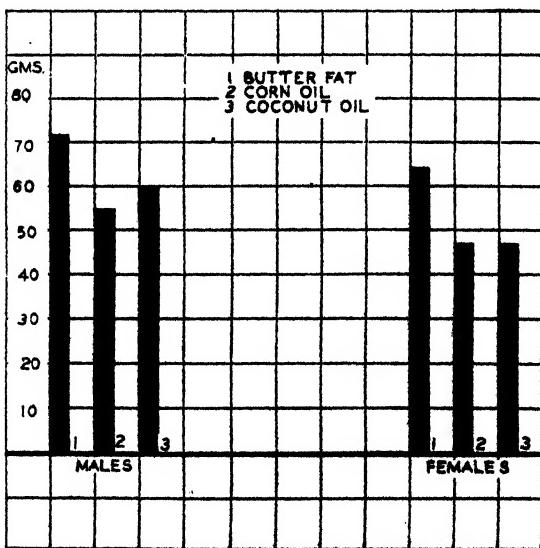


CHART III. Average gain made during the first three weeks on experiment by male and female rats representing 18 rats (9 males, 9 females) on each of the following fats: butter fat, corn oil plus non-saponifiable fraction of butter fat and coconut oil plus non-saponifiable fraction of butter fat.

terial. The animals were irradiated and treated exactly as in the experiments previously described.  $\alpha$ -Tocopherol was added to the milk once each week. Three trials have been carried out comparing butter fat with corn oil and coconut oil plus the non-saponifiable fraction of butter fat representing 18 animals on each fat. The rats on the fats with the non-saponifiable fraction of butter fat added showed no better response than the animals fed the fats without the non-saponifiable fraction. Chart III illustrates the gain made by these animals during the first three weeks on the experiments. Comparison of chart III with chart II or IV clearly illustrates that the inferior growth obtained on the corn oil and coconut oil milks is not corrected by adding the non-saponifiable fraction of butter fat to these fats.

The data on the growth and food consumption in a single experiment (experiment 8) involving the five fats are represented in chart IV and tables 1 and 2. In the case of the corn oil and coconut oil, the non-saponi-

TABLE I, EXPERIMENT 8

*Average daily gains for each week for male and female rats  
(6 animals on each fat—3 males, 3 females)*

Week	Males				
	Butter fat	Corn oil	Coconut oil	Cottonseed oil	Soybean oil
gm.	gm.	gm.	gm.	gm.	gm.
1	2.8	1.7	2.3	2.3	1.8
2	4.5	3.7	3.8	4.0	3.4
3	3.9	2.4	2.9	3.4	3.4
4	3.7	3.3	3.4	2.0	3.0
5	3.3	4.1	5.0	3.3	4.7
6	3.4	3.6	2.6	3.9	4.7
	Females				
	gm.	gm.	gm.	gm.	gm.
1	2.6	1.8	2.1	2.1	1.7
2	3.8	3.1	3.0	3.5	3.4
3	3.4	2.9	2.6	3.1	3.0
4	3.3	2.0	1.4	2.4	2.4
5	2.9	3.4	2.7	2.0	2.7
6	2.0	2.0	2.0	1.7	2.1

fiable matter of butter fat equal to that in the four per cent of butter was added. Chart IV illustrates the gain made during the first three weeks on the experiment. Table 1 shows the average daily gains made by the male and female rats during each week of the experiment. The differences in growth rate appeared most noticeable and consistent during the first three or four weeks. During this period the rats on butter fat gained from one-half to one gram more than the animals on the vegetable oils. Table 2 shows the number of cc. of milk required to produce one gram of gain in weight during each week of the experiment. With the exception of some variation

TABLE 2, EXPERIMENT 8

*Number cc. milk to produce one gram gain in weight for each week  
(6 animals on each fat—3 males, 3 females)*

Week	Males				
	Butter fat	Corn oil	Coconut oil	Cottonseed oil	Soybean oil
	cc.	cc.	cc.	cc.	cc.
1 .....	10.7	19.9	11.7	12.0	14.6
2 .....	10.5	10.8	10.8	10.9	11.0
3 .....	13.7	17.5	17.9	15.0	13.6
4 .....	18.3	16.2	20.6	20.0	19.8
5 .....	20.6	14.6	14.3	23.1	16.4
6 .....	20.0	16.6	28.3	15.2	16.5
Average at end of 3 weeks ..	11.6	16.0	13.5	12.6	13.1
Average at end of 6 weeks ..	15.6	15.9	17.2	16.0	15.3
Females					
	cc.	cc.	cc.	cc.	cc.
1 .....	10.6	14.8	12.2	11.9	15.9
2 .....	11.5	12.3	13.9	12.8	11.5
3 .....	15.1	18.0	17.9	17.3	15.6
4 .....	21.2	23.0	28.3	23.0	27.6
5 .....	22.4	18.8	20.1	26.0	20.7
6 .....	27.1	22.0	28.3	29.3	31.7
Average at end of 3 weeks ..	12.4	15.0	14.6	14.0	14.3
Average at end of 6 weeks ..	18.0	18.1	20.1	20.1	20.5

the butter fat milk appears to be utilized more economically than the other milks. This shows well in the average at the end of three weeks but the average at the end of six weeks does not show differences in all cases.

Figure 1 illustrates the differences in growth and general appearance of rats at the end of three weeks on butter fat, corn oil, and coconut oil milks. Number 1, on butter fat, gained 63 grams; number 7, on corn oil, gained 45 grams; and number 13, on coconut oil, gained 40 grams during the first three weeks of the experiment.

Since considerable difficulty was encountered in raising the pups from the females on the vegetable oils, even after  $\alpha$ -tocopherol was fed, this part of the investigation, in which all animals have received  $\alpha$ -tocopherol throughout the experiment, is being repeated.

#### DISCUSSION

While the differences in growth on the different milks were small, the growth rates of the animals on the butter fat milk were consistently greater

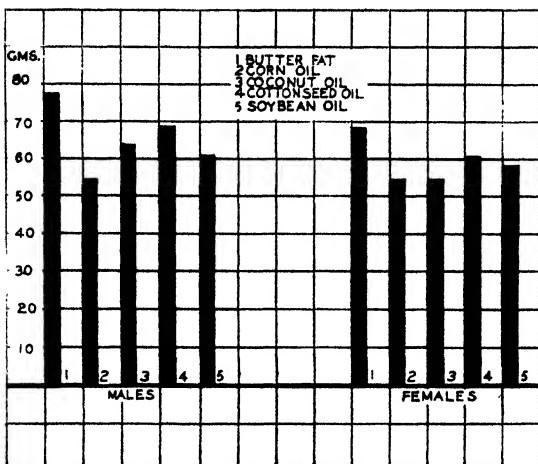


CHART IV (Exp. 8). Average gain made during the first three weeks on experiment by male and female rats representing 6 rats (3 males, 3 females) on each of the following fats: butter fat, corn oil, coconut oil, cottonseed oil, and soybean oil.

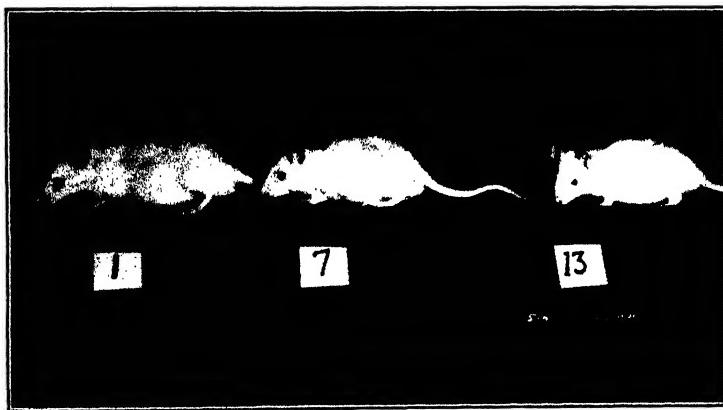


FIG. 1. Illustration of the differences in growth and appearance of rats after three weeks on the following fats homogenized into skimmed milk: butter fat (No. 1) gained 63 grams; corn oil (No. 7) gained 45 grams; and coconut oil (No. 13) gained 40 grams during the first 3 weeks of the experiment.

during the first two or three weeks of each experiment than the growth rates of the animals on the milks containing the different vegetable oils. After about three weeks these differences became less apparent. Another important observation in these experiments is that the animals on butter fat had a better general appearance and a finer coat of hair than the animals on the milks containing the vegetable oils. These points were observed even after

the non-saponifiable fraction of butter fat was added to the corn oil and the coconut oil. It appears then that the growth-stimulating property of butter fat found in these experiments lies in the saponifiable fraction of butter fat. However it is possible that the saponification process may have destroyed the growth-stimulating property but this point cannot be definitely settled until the fatty acid fraction of butter fat is fed along with the vegetable oils. The saponification was carried out at 37° C. to prevent, as much as possible, any destruction of growth factors. There is also the possibility that the change from the milk of the mother to the milks fed in these experiments is greater for the vegetable oils than for butter fat and the animals lose time in becoming accustomed to the different milks. However, in many cases the animals did well for the first week and then dropped off in growth, which points to the possibility of some deficiency of a factor necessary for optimum growth rather than adaptation to the different milks. If a deficiency of some factor does occur in the young animals on the vegetable oil milks, the animals apparently supply themselves with the factor after a time since the growth rate becomes equal to that on butter fat. The fact that corn oil did not give optimum growth indicates that the inferior growth is not caused by the lack of the now recognized essential unsaturated fatty acids.

The food consumption records of the animals on the different milks show that the butter fat milk was utilized more economically for growth than the milks containing the vegetable oils. This was true especially during the first part of the growing period. Holt (5) has reported that soybean oil is superior to butter fat in infant nutrition because this oil is more easily absorbed than butter fat. In our work soybean oil was found to be much inferior to butter fat, especially during the first week of the experiment (tables 1 and 2). However, in our work it was observed that the animals on the soybean oil milk and cottonseed oil milk had gained more weight within the first three weeks than the animals on the corn oil and coconut oil milks but still were inferior to the animals on the butter fat milk. Gullickson and Fountaine (6) found that calves about one week of age placed on certain vegetable oils homogenized into skimmed milk died after a time while calves on butter fat homogenized into skimmed milk grew normally. If we could have started our rats earlier than three weeks of age and prevented the pups from receiving the fat from the mother's milk, greater differences in weight and appearance might have resulted.

The data on growth have been treated statistically and found to be significant.

#### CONCLUSIONS

Good growth was obtained in rats on a diet of four per cent butter fat, corn oil, coconut oil, cottonseed oil, or soybean oil homogenized into mineralized skimmed milk. Sufficient quantities of all known vitamins were

supplied to each diet. However, rats on butter fat made better and more efficient gains during the first two or three weeks on the experiment than rats on the vegetable oils homogenized into skimmed milk. This growth-stimulating property of butter fat appeared to lie in the saponifiable fraction since feeding the non-saponifiable fraction along with corn oil or coconut oil did not give the same response as was obtained with butter fat. Rats raised on butter fat milk had a much better appearing coat of hair throughout the experiment than the rats raised on the vegetable oil milks.

It appears that the kind of fat in the diet is important in the nutrition of the young growing animal.

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## American Dairy Science Association Announcements

### THIRTY-FIFTH ANNUAL MEETING, PURDUE UNIVERSITY WEST LAFAYETTE, INDIANA, JUNE 24-28, 1940

#### CALL FOR TITLES AND ABSTRACTS OF PAPERS

Since the abstracts of papers to be presented at our annual meeting in June are to be published in the June number of the *JOURNAL OF DAIRY SCIENCE*, it is necessary for us to have all abstracts in our hands by April 15. Please send titles and abstracts to Dr. B. E. Horrall, Department of Dairy Husbandry, Purdue University, West Lafayette, Indiana.

#### TWENTY-YEAR INDEX

A twenty-year index is being prepared and will be published sometime during the year.

#### BACK COPIES

The Association has on hand back copies of the Journal from Volume I, No. 1, to the present with the exception of Volumes I, XV and XVII. The reprinting of these volumes is now being considered and we are interested to know whether any of our readers would be in the market for Volumes I, XV, XVII, should we have them reproduced. The cost would probably be \$6.00 per volume as it is for the other back volumes which we have.

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# JOURNAL OF DAIRY SCIENCE

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## DETERMINATION OF FAT, MOISTURE, AND SALT IN SOFT CHEESE

G. H. WILSTER, CHAIRMAN, W. V. PRICE, A. J. MORRIS,  
E. F. GOSS, G. P. SANDERS

*The Subcommittee for the Analysis of Cheese, A. D. S. A.*

Methods for determining the fat, moisture and salt contents of hard cheese were reported by the committee in 1936. They were published in Volume XX, No. 1, of the JOURNAL OF DAIRY SCIENCE.

The methods outlined below are applicable in analyses of such varieties as Limburger, Brick, Roquefort, Bel Paese, cottage, Neufchatel, cream, Camembert, etc., or such varieties as are characteristically sticky. In analyzing samples of cheese which are more firm in texture, the methods previously outlined for hard cheeses (1) are used. All analyses should be made in duplicate.

### 1. Sampling:

When the cheese can be cut, take a narrow wedge-shaped segment reaching from the outer edge to the center. If the loaf or block is small, cut it through the center and remove a slice of suitable thickness to provide a sufficient amount of sample, from the freshly cut surface. If the cheese, or the contents of a package, is too small for obtaining a slice the whole cheese or the total contents of the package must be used. Cut the sample into strips and pass through a food grinder (the preferable method), then place a representative portion in a glass tumbler. If it is necessary to save the cheese and it is not desirable to cut the cheese, in the case of some varieties, take the sample by means of a cheese trier. Remove a plug, as long as possible, from the outer edge, at a point mid-way between the top and bottom surfaces. When possible draw three plugs from different and representative points. For inspection purposes reject one-half inch nearest the rind, but for analyses requiring absolute data reject only such portion at the surface as may be inedible. If a plug sample is used, cut and mix the sample thoroughly in a tumbler by means of a spatula. In the case of some varieties which are very soft, such as cottage and Neufchatel, remove from the package, by means of a spatula, a wedge-shaped portion or a slice as described above, transferring this portion to a tumbler to be mixed thoroughly with a spatula and analyzed at once.

All samples unless analyzed immediately are wrapped in foil or similar

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non-absorbent material before being ground and are enclosed in small, air-tight, stoppered containers and analyzed as soon and as rapidly as possible without unnecessary exposure to the air. Samples not analyzed at once are kept in a refrigerator.

### 2. *Determination of fat:*

(1) Weigh exactly 9 grains of sample as prepared above into a dry, tared, 9-gram, 50 per cent, large-bodied Babcock cream test bottle. This operation should be carried out quickly. The cheese is inserted into the bottle by means of a thin-walled Pyrex glass tube 6 inches long and 11/32 inch outside diameter, and a solid glass rod 6½ inches long and about ¼ inch in diameter, or of the proper size to fit snugly, without binding, into the glass tube.\* Cheese is inserted into the glass tube by tapping into the sample with the end of the tube; for very soft cheeses, such as cottage, the tube and the tumbler containing the sample should be held in a horizontal position, while the cheese is being inserted. The outer surface of the tube is wiped several times against the inside of the top of the tumbler and then wiped clean on a paper towel. The tube is inserted into the neck of the bottle and the cheese is pushed into the bottle by means of the glass rod. The rod is wiped clean after it is removed from the tube. By this method cheese is not left sticking to the inner surface of the neck of the test bottle.

(2) Add 12 cc. water at a temperature of 160° to 170° F. Mix well with the cheese.

(3) Add in several installments 17.5 cc. sulphuric acid (sp. gr. 1.82 to 1.83), shaking the bottle after each addition of acid. Let the bottle stand until all particles of cheese have dissolved.

(4) Centrifuge and add water as when testing cream.

(5) Place the bottle in a water bath 130° to 140° F., with the water level above the level of the fat. After 5 minutes add glymol and read the percentage of fat. Duplicate samples should check within 0.5 per cent.

### 3. *Determination of moisture:*

The sample in a tumbler having been prepared as directed above, the weighing of the sample should be begun immediately and completed as rapidly as possible in order to avoid evaporation due to unnecessary exposure to the air.

#### (a) *For laboratories.*

(1) The moisture dishes and covers are dried for 1 hour at 100° C. and allowed to cool for ½ hour in a desiccator containing sulphuric acid or other desiccant.

(2) A cover is placed on the dish and both are weighed on a chemical balance, preferably chainomatic.

\* Suggested by Robert E. Hardell, U. S. D. A.

(3) Approximately 2 to 3 grams of the sample are placed in the dish, the cover is immediately replaced, and a second weighing is made. Tests should always be made in duplicate or triplicate for the greatest accuracy.

(4) The samples are dried, with covers, in an oven at 100° C. If a vacuum of 20 inches or more is available, 10 hours' drying should suffice; if no vacuum is used, 24 hours' drying is recommended. Spattering may be minimized by placing the samples in the oven when the oven temperature is below 50° C. so that the samples are heated slowly. Covered dishes must be used to avoid losses of cheese when samples are placed in a hot oven. Vacuum should be applied slowly and released slowly.

(5) After drying, the samples are placed in a desiccator for about one hour, or until they reach room temperature, and each is weighed, without further delay.

(6) Loss in weight divided by weight of sample multiplied by 100 equals percentage of moisture.

Either 30 cc. pyrex beakers or aluminum dishes approximately 50 mm. in diameter and 22 mm. deep may be used. Each dish should be plainly and permanently numbered.

For routine laboratory analysis, if a balance having a tare beam and beams for direct readings and possessing a sensibility reciprocal\* of 15 mg. is available, the above procedure is used with the following modifications:

(1) A dish 3 inches in diameter and 1 inch deep is used.

(2) Exactly 10 grams of the freshly-prepared sample are quickly weighed into the dish.

(3) A cover is placed loosely on the dish. After being dried in an oven and cooled in a desiccator, the dish and cover are placed on the balance, and the percentage of moisture is read on the beams to the nearest 0.1 per cent. Duplicate samples when properly dried should check within 0.2 per cent.

(b) For cheese factories.

(1) Tare a dry, aluminum dish with cover on a balance which is equipped with a tare beam and with beams that permit the direct reading of the percentage of moisture. The balance should have a sensibility reciprocal of 15 mg. If the dish is first heated in order to dry it, cooling it afterwards to room temperature is important. A dish 3 inches in diameter and about 1 inch deep is satisfactory.

(2) From the freshly prepared sample weigh 10 grams cheese into the dish. This operation should be done quickly. The lid is placed loosely on the dish so as to permit the escape of moisture. The lid prevents the escape of fat and casein if spattering occurs.

(3) The dish is placed in an oven and heated slowly to a temperature

\* The pointer should be deflected a distance equal to one division on the graduated portion when 15 mg. are placed on either scale pan when the scale is loaded to capacity.

of 220° to 230° F. This temperature should be maintained for 24 hours. An electrically heated oven equipped with a heat regulating device is satisfactory for this purpose. If electricity is not available, a pressure steam oven may be used. Since steam is usually not available over a period of 24 hours in the average cheese-factory, with a steam pressure in the jacket of the oven of from 40 to 50 pounds, and a temperature in the oven of 290° F., the drying can be completed in 4 to 6 hours. The temperature should be increased over a period of 1 hour to that desired. This procedure will avoid boiling over of the cheese. Electrically heated ovens can also be used for this short drying treatment.

(4) When the dish is removed from the oven the lid is placed tightly on the dish and the dish with moisture-free material is placed on a cool surface to cool to room temperature. The dish is then placed on the balance and the percentage of moisture read on the beams to the nearest 0.1 per cent after equilibrium has been reached. Duplicate samples should check within 0.2 per cent.

#### 4. *Determination of salt (sodium chloride):*

Weigh accurately approximately 3 grams of the prepared sample of cheese into a 300 cc. Erlenmeyer flask and add 10 cc. of 0.1711 *N* silver nitrate solution, (prepared by using 29.06 grams of C.P. silver nitrate ( $\text{AgNO}_3$ ) and making up to one liter in distilled water) or an amount more than sufficient to combine with all of the chlorine. Add 15 cc. of halogen-free, chemically pure nitric acid and 50 cc. of water and boil. As the mixture boils add approximately 15 cc. of saturated potassium permanganate solution in 5 cc. portions. Boil until all cheese particles are digested. Dilute the solution to about 100 cc., decant off the liquid into a beaker, and wash the precipitate by adding 100 cc. of water and decanting again. Add 3 cc. of a saturated solution of ferric ammonium sulfate as an indicator and titrate the excess silver nitrate with 0.1711 *N* potassium or ammonium sulfocyanate (prepared by dissolving 16.63 grams C.P. potassium sulfocyanate and making up to one liter in distilled water). Run a blank on the reagents used, following the same procedure, except to add sugar to destroy the excess of permanganate. The number of cc. of silver nitrate used minus the titration value divided by the weight of sample equals the percentage of sodium chloride in the sample. The reagents should be standardized against a salt solution containing 10 grams of chemically pure, dry sodium chloride per liter.

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# OXIDIZED FLAVOR IN MILK: I. EFFECT OF THE DEVELOPMENT OF OXIDIZED FLAVOR ON THE IODINE NUMBER OF THE PHOSPHOLIPID FRACTION OF MILK

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Oxidized flavor in milk and milk products is generally accepted to be the result of oxidation of one or more of the lipids present in milk. The oxidation of the unsaturated fatty acids present in butterfat was originally thought to be the source of oxidized flavor, but the evidence presented by certain workers shows that the phospholipids present in milk may play an important rôle.

The work reported herein was undertaken to determine which of the lipid constituents in milk had undergone a change on the development of oxidized flavor. The oxidized flavored milk used in these experiments was obtained by the addition of copper in the form of copper sulfate solution. Unfortunately there was no milk available which would develop the off flavor spontaneously.

## REVIEW OF LITERATURE

Thurston (13) states that three classes of milk must be recognized when oxidized flavor is studied. He suggests that the following names and classifications be used: 1, *spontaneous milk*—milk which will develop oxidized flavor without any added metallic catalyst; 2, *susceptible milk*—milk which requires the addition of copper or iron to cause the development of oxidized flavor; and 3, *non-susceptible milk*—milk in which oxidized flavor cannot be produced by the addition of a metallic catalyst. The above classifications will be used in this paper when referring to the different classes of milk.

Evidence was presented by Thurston, Brown and Dustman (14) to indicate that the phospholipid fraction of milk rather than the butterfat was the substance which had undergone oxidation on the development of oxidized flavor. These workers found that the intensity of the oxidized flavor in cream, skimmilk, buttermilk and butter obtained from oxidized milk was in direct relation to their phospholipid content. Remade milk, in which washed cream from oxidized milk was incorporated into normal skimmilk, did not have an oxidized flavor. The addition of copper to remade milk in which butterfat from normal milk was used did not develop an oxidized flavor. Tallowy butterfat incorporated into skimmilk produced a milk having a flavor differing from the typical oxidized flavor. These findings would indicate that the phospholipids which are removed by washing the butterfat play an important rôle in the development of oxidized flavor.

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Horrell (7) found skimmilk to contain about half the amount of lecithin contained in the original whole milk. Thurston, Brown and Dustman (14) use this fact that skimmilk does contain lecithin to explain the development of oxidized flavor in skimmilk.

Chilson (3), working with spontaneous milk, also found that milk remade from washed cream would not develop an oxidized flavor. The addition of small amounts of copper sulfate to this remade milk would not cause the oxidized flavor to develop, but the addition of 30 p.p.m. of copper produced a tallowy flavor.

Dahle and Palmer (4) substantiate the findings of Thurston, Brown and Dustman (14) in regard to the development of oxidized flavor in susceptible milk, but they found that on the addition of washed cream to skimmilk from spontaneous milk that the remade milk developed an oxidized flavor.

Roland and Trebler (11) standardized milks and after exposing these milks to copper, found that the higher the percentage of fat, the more intense was the oxidized flavor. They also suggest that lecithin may be a factor in the development of oxidized flavor. Horrell (7) found that as the amount of butterfat in milk increased so did the amount of lecithin present increase.

Beck, Whitnah and Martin (1) found no relation between the frequency of occurrence of oxidized flavor and the lecithin content of raw milk.

Thurston, Brown and Dustman (15) observed that homogenization, prolonged agitation at low temperatures, and freezing and thawing of milk caused the milk to become less susceptible to the development of oxidized flavor. The explanation given for the above results is that lecithin is transferred from a film around the fat globules into the serum, and being in a more dilute form and more widely dispersed, it is not so readily oxidized. Tracy, Ramsey and Ruehe (16) and Ross (12) have also found that homogenization makes milk less susceptible to oxidized flavor.

Kende (8) has shown that the iodine number of butterfat decreases with the development of oxidized flavor due to the addition of a metallic catalyst to the milk. He also found that when milks developed the flavor spontaneously there was a decrease in iodine number but not as large as with metal contamination. Dahle and Palmer (4) observed a decrease in the iodine number of butterfat when oxidized flavor developed spontaneously. Brown, Dustman and Thurston (2) could find no appreciable difference due to copper contamination in iodine number of butterfat from normal milk and from oxidized milk.

From the work of Henderson and Roadhouse (5) milk to which copper had been added developed oxidized flavor, but the butterfat had passed only a fraction of its induction period. Holm and Greenbank (6) report that the iodine number of butterfat does not decrease until after the end of the induction period has been reached and the oxygen absorption is on a rapid increase.

#### EXPERIMENTAL

The object of these experiments was to compare the iodine numbers of the phospholipid fractions from normal milks with those from oxidized milks and also to compare the iodine numbers of butterfat from the same milks. After some preliminary experiments, the following procedure was adopted for obtaining iodine numbers of the phospholipid fraction.

##### *Experiment 1*

Ten gallons of milk from the University Creamery intake was pasteurized at 62.2° C. for 30 minutes in two aluminum milk cans. The milk was then immediately cooled to 32° C. and divided into two equal lots. Lot A was separated in a new hand separator and the cream cooled and held at 0° C. To lot B copper sulfate solution was added at a concentration of 3 p.p.m. of copper, and then the milk was cooled to 4° C. and held at that temperature for three days.

The cream from lot A, after being held at 0° C. for 3 hours, was churned in stainless steel laboratory churning cans. The buttermilk was used as the source of the phospholipid fraction. Four liters of buttermilk were extracted by the Rose-Gottlieb ether extraction method. The ether was then removed from the lipid residue by distillation under vacuum at a temperature of not over 30° C. The distillation was carried on in a complete glass distilling apparatus, and the distillate receiving vessel was packed with dry ice. The vacuum was obtained by means of a mechanical pump.

Immediately upon the removal of the ether from the lipid residue, 500 cc. acetone at 24° C. was added. The phospholipids, lecithin and cephalin, are insoluble in the acetone and form a flocculent white precipitate. The acetone containing the butterfat in solution was removed by decantation. The precipitate was then rewashed with acetone until the acetone remained colorless. The precipitate was dried under vacuum and, when dry, immediately taken up in 60 cc. of chloroform.

Hanus iodine number determinations were made in duplicate on 10 cc. samples of the chloroform solution. The weight of the phospholipids in 10 cc. of the sample was obtained by determination of dry material on evaporation of the chloroform.

The acetone containing the rest of the lipid residue was held at 0° C. for 10 hours. Further precipitation of lipid present in the acetone solution occurred. This precipitate was handled in similar manner to the previous precipitate and duplicate Hanus iodine number determinations were made.

The acetone was also removed from the remaining soluble lipid fraction by means of vacuum distillation and iodine number determinations were made on this residue. A sample of butter oil prepared from the butter obtained in churning was used for Hanus iodine number determinations on butterfat. The peroxide number and the index of refraction were also determined on the last two samples.

The milk in Lot B at the end of the three day storage period had developed a distinct oxidized flavor.

The milk was warmed to 32° C. and separated. The cream was then handled in the identical manner as was the cream in Lot A. Care was taken in the separation and analysis of the lipid fractions so that all conditions were the same.

### *Experiment 2*

This experiment was conducted in a manner similar to experiment 1 for the purpose of verifying the previous results. Instead of starting with whole milk, six gallons of raw cream containing 30 per cent of butterfat from the separator in the University Creamery was used. The cream was pasteurized at 62.2° C. for 30 minutes in an aluminum milk can and then cooled to 32° C. The cream was divided into two equal lots. Lot A was immediately cooled to 4° C. and held 10 hours before churning. Three p.p.m. of copper in the form of copper sulfate solution was added to lot B, and it was then cooled to 4° C. and held at this temperature 72 hours before churning.

After churning, the two lots were handled in the identical manner as the two lots in experiment 1, but only the phospholipid fraction was removed from the ether soluble fraction for the iodine number determinations. Hanus iodine number determinations were made on samples of butter oil prepared from the butter obtained on churning of the two lots of cream.

Experiment 1 was conducted during the first week of July 1938 and experiment 2 during the third week of April 1939.

### EXPERIMENTAL DATA

Table 1 summarizes the data obtained in experiment 1. The values for iodine numbers on the different lipid fractions are averages of the duplicate determinations.

TABLE 1

*The effect of the development of the oxidized flavor on the fat constants of the different fat fractions from normal and oxidized milk*

Lipid fraction	Source of lipid fraction	Fat constants		
		Iodine No.	Refractive index 40° C.	Peroxide No.
Fraction insoluble in acetone at 24° C. (lecithin and cephalin)	{ Normal milk Oxidized milk	60.34 33.53		
Fraction soluble in acetone at 24° C. but insoluble at 0° C.	{ Normal milk Oxidized milk	20.47 32.15		
Fraction soluble in acetone at 0° C. ....	{ Normal milk Oxidized milk	52.58 52.52	1.450 1.388	0.0 trace
Butter-oil .....	{ Normal milk Oxidized milk	47.96 48.50	1.419 1.418	0.0 trace

Table 2 gives a summary of the iodine number determinations made on the phospholipid fraction (lecithin and cephalin) of normal and oxidized milks and on the butter-oil samples obtained from the same milks in experiment 2.

TABLE 2

*The effect of the development of oxidized flavor on the iodine numbers of the phospholipid fractions and of samples of butter-oil from normal and oxidized milk*

Lipid fraction	Source of lipid fraction	Iodine number
Fraction insoluble in acetone at 24° C.	{ Normal cream } Oxidized cream	48.72 33.67
Butter-oil	{ Normal cream } Oxidized cream	33.12 32.82

## DISCUSSION

Evidence in the literature suggests that the lecithin in milk rather than the butterfat becomes oxidized during the development of oxidized flavor. No chemical evidence has been given to substantiate the above assumption outside of the work of Brown, Dustman and Thurston (2). These workers could find no appreciable difference in the iodine number of butterfat from normal milk as compared to butterfat from oxidized milk. They also suggest that if oxidation of the lecithin had occurred there would not have been a marked change in the iodine number of the butterfat due to the small quantity of lecithin present. From the work of Kende (8) and Dahle and Palmer (4) the butterfat must also undergo oxidation when the oxidized flavor develops spontaneously in milk to which no metallic catalyst has been added.

The phospholipids, lecithin and cephalin, are insoluble in acetone at room temperature and will form a white flocculent precipitate, while the rest of the lipid fraction is quite soluble. This property of the phospholipids, lecithin and cephalin, was used in removing them from ether soluble residues obtained from normal and oxidized buttermilk. Lecithin and cephalin are soluble in chloroform, and chloroform solutions of the phospholipids were used for the Hanus iodine number determinations.

In this work no attempt was made to separate the cephalin from the lecithin, so the reported results indicate changes in the phospholipid fraction containing lecithin and cephalin. The results may then be due to oxidation occurring in the lecithin or in the cephalin or in both substances. Kurtz, Jamieson and Holm (9) by means of titration with sodium hydroxide found that purified glycero-phosphatides of milk consist of 44 per cent cephalin and 56 per cent lecithin. Rewald (10) in a recent paper found butter phosphatides have approximately the following composition: 36 per cent cephalin (alcohol insoluble), 50 per cent lecithin (alcohol soluble) and a

14 per cent fraction which is soluble in hot alcohol and insoluble in cold alcohol.

Brown, Dustman and Thurston (2) calculated the theoretical iodine number of a lecithin which contains one saturated fatty acid, stearic, and one unsaturated fatty acid, oleic, to be 31.54. At one time it was generally assumed that milk lecithin contained one saturated and one unsaturated fatty acid; this assumption has since been found to be untrue. Kurtz, Jamieson and Holm (9) assumed that there is a mixture of fatty acids in milk phospholipids and that oleic acid represents 70.0 per cent of the fatty acids present. They also state that there are none of the lower fatty acids in the glycerophosphatides of milk.

From experiments 1 and 2 the iodine numbers of the phospholipid fraction from oxidized milk were 33.53 and 33.67 respectively. The oxidation of the phospholipid fraction is not complete and the final iodine numbers are quite close to the theoretical iodine number of 31.54 for lecithin assuming stearic acid and oleic acid, so it would seem that one oleic acid molecule in each molecule of lecithin remained unoxidized. The iodine number of the phospholipid fraction from the normal summer milk in experiment 1 was 60.34 and for the normal early spring milk in experiment 2 was 48.72. Both of these iodine numbers were considerably higher than those for phospholipid fractions of oxidized milk, which indicates the presence of more than one unsaturated fatty acid in each molecule of lecithin. The phospholipid fraction from summer milk contains more unsaturated fatty acids than the phospholipid fraction from early spring milk. Apparently the presence of natural reducing substances in summer milk tends to inhibit the development of oxidized flavor even though the iodine number is higher.

In both experiment 1 and experiment 2, the milk to which copper had been added developed typical oxidized flavor. In experiment 1 there was a 44.41 per cent reduction in iodine number of the phospholipid fraction, and in experiment 2, a 30.89 per cent reduction in iodine number of the phospholipid fraction on the development of oxidized flavor.

There was no significant difference between the iodine numbers obtained on samples of butterfat from normal and oxidized milk. In experiment 1 there also appeared little difference in the refractive indices of butterfat from normal and oxidized milk. The sample of butterfat from oxidized milk showed a trace of peroxide formation but none was observed in the sample of butterfat from normal milk.

In experiment 1 the lipid fraction soluble in acetone at 24° C. and insoluble at 0° C. evidently contains some of the fats, particularly the ones containing saturated fatty acids because the iodine number is lower than for the regular butterfat. Fats are known to be insoluble in acetone at low temperature. The fraction from normal milk is lower in iodine number

than the fraction from oxidized milk. This difference may have a significant relation to oxidized flavor development but no explanation is apparent.

The lipid fraction soluble in acetone at 0° C. showed little difference in iodine number between that obtained from normal milk and oxidized milk. The sample from oxidized milk showed a slight decrease in refractive index and a trace of peroxide formation. The decrease in refractive index of the fraction from oxidized milk may also have significant relation to the development of the oxidized flavor.

These experiments were conducted in a dark room to minimize the effect of light on the phospholipid fractions, which are known to be readily oxidized when exposed to light and air. Small samples of the phospholipid fraction from normal and oxidized milk were dried and then observed for appearance. The sample from normal milk was white and flaky on drying, but the sample from oxidized milk had a greenish tint and was greasy in appearance. Neither sample had an odor or taste which resembled the oxidized flavor of milk.

#### SUMMARY AND CONCLUSIONS

The Hanus iodine numbers of the phospholipid fractions from normal and oxidized milk were determined. The development of oxidized flavor is accompanied by a marked decrease in the iodine number of the phospholipid fraction. Iodine number determinations on samples of butterfat from normal and oxidized milk showed no significant difference. The conclusion can be drawn that the development of oxidized flavor in milk, catalyzed by copper, is primarily due to the oxidation of the phospholipid fraction.

The oxidation of the unsaturated fatty acid in the phospholipid fraction is not complete, but the indications are that one molecule of an unsaturated fatty acid, undoubtedly oleic acid, remains unoxidized.

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# EFFECT OF CONDENSING ON THE DEVELOPMENT OF OXIDIZED FLAVOR

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In previous work done at this station by Tracy (1) on the manufacture of bottled concentrated whole milk, it was observed that the oxidized flavor which frequently occurred in regular milk did not develop in the concentrated milk, even though no effort was made to exclude air from the samples after condensing. In 1938, Guthrie, Hand and Sharp (2) reported that the destruction of ascorbic acid and the development of oxidized flavor could be largely or completely prevented by the removal of oxygen from the milk by treating under vacuum. The following study was made to determine more exactly the role of the condensing process in the prevention of the oxidized flavor.

## METHODS

All samples of whole milk were standardized to four per cent fat and then pasteurized by heating to 143° F. for 30 minutes in a stainless steel vat. The milk was condensed in either a small laboratory glass condensing unit or in a three-foot stainless steel condensing pan.<sup>1</sup> The condensing was done at a vacuum of 24–25 inches at 135–140° F. By means of a double valve arrangement in the pan outlet it was possible to take samples during the condensing process without shutting down. Samples were cooled immediately to approximately 40° F. and later reconstituted to four per cent fat with tap water. To a part of the samples 3 p.p.m. of copper were added in a 1 per cent solution of copper sulfate. The milk samples were held at 40° F. and judged for degree of oxidized flavor at various time intervals.

## CONDENSING AS A RETARDER OF OXIDIZED FLAVOR

Several lots of four per cent fat milk were pasteurized and condensed to a 2-1 concentration. The effectiveness of condensing as a retarder of oxidized flavor is shown by the data in table 1, which is typical of many trials.

The data show conclusively that the reconstituted condensed milk did not develop the oxidized flavor, even when 3 p.p.m. of copper was added. In these trials no precautions were taken to exclude air from the condensed milk after it was taken from the vacuum pan. In several experiments compressed air was bubbled through the reconstituted condensed milk to which copper had been added for several minutes, and yet the oxidized flavor did not develop during holding periods of several days.

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<sup>1</sup> Although all the data given in this paper were taken from experiments in which the large vacuum pan was used, substantially the same results were obtained with the small laboratory condensing unit.

TABLE 1  
*Effectiveness of condensing in retarding the development of oxidized flavor*

Sample	Degree of oxidized flavor after:		
	1 day	2 days	3 days
1. Pasteurized—unconcentrated (4% fat) .....	—	—	—
2. Pasteurized—unconcentrated (4% fat) plus 3 p.p.m. copper .....	+++½	+++++	++++++
3. Reconstituted condensed milk (4% fat) .....	—	—	—
4. Reconstituted condensed milk (4% fat) plus 3 p.p.m. copper .....	—	—	—

**AMOUNT OF CONDENSING NECESSARY TO RETARD OXIDIZED FLAVOR**

Twelve hundred pounds of 4 per cent fat milk were pasteurized and drawn into the vacuum pan to determine how much condensing was necessary to protect the samples against the development of oxidized flavor. Samples of the partially condensed milk were taken at 15-minute intervals during the condensing process. The results are given in table 2.

TABLE 2  
*Relation of extent of concentration to development of oxidized flavor in reconstituted,  
condensed milk*

Sample	Fat test before reconst.	Degree of oxidized flavor after:					
		1 day		2 days		3 days	
		No cu	3 p.p.m. cu	No cu	3 p.p.m. cu	No cu	3 p.p.m. cu
1. Past. ....	4.0	—	+++	—	+++++	—	++++++
2. Cond. 15 min. reconstituted to 4% fat .....	5.0	—	—	—	—	—	++½
3. Cond. 30 min. reconstituted to 4% fat .....	6.05	—	—	—	—	—	±
4. Cond. 50 min. reconstituted to 4% fat .....	8.50	—	—	—	—	—	—
5. Cond. 50 min. Homogenized at 2500 pounds pres- sure, reconsti- tuted to 4% fat .....	8.50	—	—	—	—	—	—

It is apparent that rendering the condensed milk immune from developing the oxidized flavor involves more than simply a removal of oxygen as one would expect the dissolved air to be removed within a few minutes after the condensing process was started. As shown in these trials it is necessary to condense to practically a 2-1 concentration to entirely protect the milk from developing an oxidized flavor, suggesting that a physical change in the

serum constituents may be responsible for the protection of the fat against oxidation.

**MILK MADE FROM CREAM AND CONDENSED SKIMMILK IMMUNE TO  
OXIDIZED FLAVOR**

The following experiment was performed to determine if condensing skimmilk would be effective in preventing an oxidized flavor in milk made from cream and condensed skimmilk. Skimmilk was pasteurized by heating to 143° F. for 30 minutes and then concentrated to 30 per cent solids. Four per cent fat milk was made from 32 per cent cream, and condensed skimmilk and water. A control sample containing 4 per cent fat was made from the same lots of 32 per cent cream and the skimmilk. The results are given in table 3.

TABLE 3

*Effect of concentrating the plasma portion of milk upon oxidized flavor development*

Sample	Degree of oxidized flavor after:		
	1 day	2 days	3 days
1. 4% milk made from cream and skimmilk			
4% milk made from cream and skimmilk + 3 p.p.m. Cu	+1		
2. 4% milk made from cream, condensed skimmilk and water	-		
4% milk made from cream, condensed skimmilk and water + 3 p.p.m. Cu	-		

These data show that the effect of the condensing process in retarding oxidized flavor development is upon the plasma portion of the milk. It is possible that the condensing process causes a shift in the oxidation-reduction potential to the reduced side by the liberation of reducing substances or by the liberation of substances which act as antioxidants.

**EFFECT OF CONDENSING ON THE CURD TENSION OF MILK**

There are several instances in which factors which retard or prevent tallowy flavors in milk also reduce the curd tension of the milk. Examples of this are homogenization, addition of certain enzymes, addition of sodium salts, and high heat treatment.

Curd tension measurements were made on the samples of reconstituted condensed milk to determine the effect of condensing on the hardness of the curd. The tentative procedure adopted by the American Dairy Science Association Subcommittee on Curd Tension Measurements in 1938 was followed, and a Submarine Signal curd tension machine was used to make the measurements. The results are given in table 4. Condensing either whole or skimmilk and then reconstituting to the original solids content lowered the curd tension. The more condensing the milk was subjected to, the greater was the influence on the curd tension.

TABLE 4

*Effect of condensing on the curd tension of milk (condensed milk reconstituted with water to 4 per cent fat)*

Part A. Using condensed whole milk		
Sample	Fat test before reconstituting	Curd tension on reconstituted milk (grams)
1. Pasteurized .....	4.0	43.5
2. Cond. 15 min. ....	5.0	45.0
3. Cond. 30 min. ....	6.05	40.5
4. Cond. 50 min. ....	8.5	35.0
5. Same as 4.—Homogenized at 2500# pressure ..	8.5	22.0

Part B. Using condensed skimmilk plus cream		
1. Past. skimmilk (9.2% T.S.) .....		55
2. Cond. skim 30% T.S. reconstituted to 9.2% T.S. with water .....		33
3. 32% cream and skimmilk to make 4% fat milk .....		36
4. 32% cream and cond. skimmilk and water to make 4% fat milk .....		22

#### EFFECT OF CONDENSING ON THE RATE OF DESTRUCTION OF ASCORBIC ACID

Several investigators have shown that the oxidation of ascorbic acid (Vitamin C) in milk precedes that of fat oxidation. Guthrie, Hand and Sharp (2) have shown that evacuating hot milk and the subsequent storing in bottles under a vacuum prevents the destruction of ascorbic acid, as well as the development of oxidized flavor.

The following experiment was performed to determine the effect of condensing on the destruction of ascorbic acid. Twelve hundred pounds of 4 per cent fat milk were pasteurized at 143° F. for 30 minutes and condensed under a vacuum of 24–25 inches to a concentration of 8.2 per cent fat. Samples were obtained 20 minutes after the condensing process had started and again 25 minutes later when the milk had been concentrated 2 to 1. The relation of condensing to the oxidation of ascorbic acid and the development of the oxidized flavor are shown by the data in table 5. Sharp's (3) procedure was followed for determining the ascorbic acid content.

Apparently there is no relation between the oxidation of ascorbic acid and the development of oxidized flavor in reconstituted condensed whole milk. In the case of the control milk containing copper and the 2-1 condensed milk reconstituted to 4 per cent fat to which copper was added, the ascorbic acid was oxidized at approximately the same rate; however, the control milk developed a strong oxidized flavor, and the condensed reconstituted milk was entirely free from oxidized flavor during the entire holding period. If, as Sharp contends, there is a relationship existing between the oxidation of ascorbic acid and the development of the oxidized flavor, the mere absence of the ascorbic acid does not mean that the oxidized flavor will necessarily develop.

TABLE 5  
*Effect of condensing on rate of destruction of ascorbic acid and development of oxidized flavor*

Sample	Per cent fat	Ascorbic acid content (mgs. per liter)			Oxidized flavor after		
		Fresh	1 day	2 days	3 days	1 day	2 days
Regular pasteurized milk Past. + 3 p.p.m. Cu	4.0	18.2 18.2	14.7 < 1.0	12.8 < 1.0	10.3 < 1.0	- ++	- +++
Cond. 20 min.	5.5	18.9 (13.7)*	6.60 (4.80)	1.1 (< 1.0)	< 1.0 (< 1.0)	- -	- -
Cond. 20 min. + 3 p.p.m. Cu	5.5	18.9 (13.7)	1.38 (1.00)	1.66 (1.22)	1.25 (< 1.0)	- ++*	+ ++
Cond. 20 min. and reconst. to 4% fat	4.0	13.8	7.48	1.43	< 1.0	-	-
Cond. 20 min. and reconst. to 4% fat + 3 p.p.m. Cu	4.0	13.8	< 1.0	< 1.0	< 1.0	±	+
Cond. approx. 2-1	8.2	36.4 (16.8)*	34.3 (15.9)	28.1 (13.0)	20.8 (9.65)	-	-
Cond. 2-1 + 3 p.p.m. Cu	8.2	36.4 (16.8)	12.0 (5.5)	10.4 (4.82)	6.97 (3.23)	-	±
Cond. 2-1 and reconst. to 4.0% fat	4.0	16.6	13.20	9.03	6.45	-	-
Cond. 2-1 and reconst. to 4.0% fat + 3 p.p.m. Cu	4.0	16.6	< 1.0	< 1.0	< 1.0	-	-

\* Numbers in parentheses refer to values calculated on a 4% fat basis.

POSSIBLE EXPLANATION FOR EFFECT OF CONDENSING PROCESS ON THE  
RETARDATION OF AN OXIDIZED FLAVOR

As has been mentioned previously, there are several instances in which factors which affect the milk plasma, as shown by reductions in curd tension, also retard the development of oxidized flavor. It is entirely possible that these factors which lower the curd tension also tend to partially break down the milk protein. Possibly some of the amino acids which act as antioxidants and prevent the development of oxidized flavor are liberated during these processes. Corbett and Tracy (4) have studied the antioxidative effect of several amino acids or their esters, and found tyrosine, tyrosine ethyl ester, leucine N-ethyl ester and glutamic diethyl ester to be effective antioxidants in milk. Skimmilk powder has also been found to have antioxidative properties. The results of this study are not in agreement with those of Guthrie, Hand and Sharp (2), who explain the effect of vacuumizing milk in retarding oxidized flavor development as one of oxygen removal. It seems that the explanation needs to take into consideration possible changes in the protein complexes of the plasma portion rather than the physical removal of air or oxygen, since the introduction of air after condensing does not cause the development of the oxidized flavor. Additional studies are being made to obtain further proof for this hypothesis.

CONCLUSIONS

1. Condensing milk under vacuum to a concentration of approximately 2-1 was found to prevent the development of oxidized flavor in both the condensed milk and condensed milk reconstituted to the original solids concentration.
2. Four per cent fat milk made from condensed skimmilk and 32 per cent cream did not develop an oxidized flavor, even when as much as 3 p.p.m. of copper were added.
3. The effect of condensing in retarding oxidized flavor development is thought to be due to the liberation in the serum portion of the milk certain antioxidative constituents that are probably derivatives of the milk protein.

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# THE INFLUENCE OF "WHITE-METAL" COPPER-NICKEL ALLOYS ON THE FLAVOR OF MILK

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As shown by investigations at many experiment stations, copper contamination from dairy equipment is the principal cause of oxidized flavor in milk; furthermore, chrome-nickel iron alloy or stainless steel of the 18-8 series is not corroded by milk, and its use is desirable if oxidized flavor from metal sources is to be avoided.

Since technical difficulties have been encountered in fabricating certain parts of dairy equipment from stainless steel, manufacturers have sought alloys more easily cast and machined. This has been particularly true in the making of certain fittings, valves, and bearings. Most of the alloys developed have copper and nickel as their base, and several other elements have been added to give the alloy the desired properties—namely, the ability to make a sound casting, to be machined easily, to remain bright after use, and to leave unimpaired the flavor of milk. Copper-nickel alloys have been used in the dairy industry for many years, and those previously studied have been found to cause oxidized flavor (1, 2). Some of the "white-metal" copper-nickel alloys recently developed include the addition of other elements that apparently modify their corrosion rate in milk. The present study reports tests conducted with certain of these alloys.

TABLE I  
*Composition of the alloys tested*

Alloy No.	Percentage composition							
	Copper	Nickel	Tin	Lead	Zinc	Iron	Manganese	Chromium
A . . .	65.6	31.0	...	2.0	...	Trace	1.0	0.5
B . . .	65.0	20.0	8.0	2.0	3.0	2.0	...	...
C . . .	62.0	20.0	8.0	2.0	3.0	2.0	...	3.0
D . . .	66.0	20.0	3.0	4.0	4.0	2.5	0.5	...
E . . .	63.0	20.0	3.0	4.0	4.0	2.5	0.5	3.0
F . . .	66.5	31.0	...	2.0	...	Trace	Trace	0.5
G . . .	65.5	31.0	...	2.0	...	Trace	1.0	5.0

## EXPERIMENTAL PROCEDURE

*Source of milk.* The milk used in these studies was obtained from normal cows of the Station herd producing milk of good flavor. All the cows received identical rations. The milk was drawn into new, well-tinned pails

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and was poured immediately into amber bottles in ice water. All surfaces of the pails and glass utensils coming in contact with the milk were rinsed with distilled water to prevent the addition of copper from tap or boiler water.

*Determination of corrosion rate.* The milk was pasteurized in contact with thin strips of the cast alloys 1 inch by 2 inches in size. These were cleaned, dried, and weighed before and after pasteurization according to the procedure previously described by Guthrie, Roadhouse, and Richardson (2). The alloys were agitated in the milk during the heating, holding, and cooling procedures, which required approximately 1½ hours. The pasteurization temperature was 142° F.

The averages of the corrosion rates reported in table 2 are for eight determinations.

*Scoring the milk.* The processed samples, together with control samples of the same milk, were held at 40° F and scored for flavor by two judges after storage periods of two to four days. The controls and test samples were arranged in random order in duplicates and scored as unknowns. Milk samples reported as having definitely oxidized flavor were detected by both judges in the duplicate samples.

*Determination of ascorbic acid.* Ascorbic acid was determined by a modified Bessey and King titration. The acid solution used for precipitating the protein and for controlling the pH of the serum to be titrated consisted of 15 per cent trichloracetic acid plus 2 per cent meta-phosphoric acid. First 15 ml. of the acid solution was added to 25 ml. of milk. After standing for a few minutes with frequent shaking, the mixture was filtered. Then 10 ml. of the clear serum was titrated with approximately 0.2 per cent sodium 2,6 dichlorobenzene indophenol solution held in a micro burette. The end point was the first permanent light-pink color that persisted for 30 seconds as determined by a stop watch. The dye was standardized by the thiosulfate method of Menaker and Guerrant (3). The first ascorbic-acid determinations were made immediately after the completion of the experimental treatment of the milk, and subsequent determinations followed various storage periods at 40° F.

#### EXPERIMENTAL DATA

*Rates of corrosion.* Typical data secured on the rates of corrosion of the alloys by milk during the pasteurization process appear in table 2. The weight losses of the alloys obtained from eight tests are presented in table 3. The losses are reported in milligrams per square decimeter per day in order that comparisons may be made with values reported, in the literature, for other metals. Alloys A, F, and G were noticeably tarnished after exposure to milk, whereas the others remained bright.

*Influence on milk flavor.* In studying the development of copper-induced

oxidized flavor, one must consider the differences in susceptibility of the milk produced by the individual cows to develop this flavor defect. Eight cows were used in this experiment; and from other experiments not reported in this paper, five were classified as "susceptible" and three as less "susceptible" to the development of copper-induced oxidized flavor. The "susceptible" cows produced milk that developed the flavor when minute quantitites of cupric ion were present, whereas the less susceptible ones required appreciable amounts of cupric ion (approximately 0.5 p.p.m.). The variation in the susceptibility of milk of individual cows to develop oxidized flavor

TABLE 2

*Variation in the susceptibility of milk of different cows to develop oxidized flavor*

Cow No.	Alloy sample No.	Days stored at 40° F.			Mlg. weight loss during pasteurization
		2	3	4	
181	Control .....	-	-	-	.
	A .....	+	+	+	2.18
	B .....	-	+	+	0.60
	C .....	-	+	+	0.74
	D .....	-	+	+	.
	E .....	-	+	+	2.43
	F .....	+	+	+	2.40
	G .....	+	+	+	2.83
498	Control .....	-	-	-	.
	A .....	-	±	+	2.79
	B .....	-	-	-	0.39
	C .....	-	-	-	+ 0.17
	D .....	-	-	-	0.86
	E .....	-	-	-	2.20
	F .....	-	±	+	1.40
	G .....	-	±	+	3.24

- No oxidized flavor or odor.

± Oxidized flavor or odor.

† Judges not in agreement as to flavor.

TABLE 3

*Corrosion rates and influence of alloys on flavor of milk*

Alloy No.	Average of eight trials		
	Weight loss calculated as mlg/dm <sup>2</sup> /day†	Percentage* of samples having oxidized flavor after storage at 40° F.	
		2 days	3 days
A .....	117.0	87.7	100.0
B .....	26.6	37.5	62.6
C .....	14.2	37.5	62.6
D .....	29.4	37.5	50.0
E .....	143.0	37.5	62.6
F .....	114.0	75.0	100.0
G .....	186.0	87.7	100.0

\* 5 susceptible cows, 3 less susceptible cows.

† milligram per square decimeter per day.

when exposed to copper-nickel alloys of different compositions is illustrated in table 2. Cow 181 is classified as susceptible, whereas cow 498 is less susceptible. The percentages of samples having oxidized flavors after 2 and 3 days' storage at 40° F are shown in table 3. Here again are illustrated the differences in susceptibility of the milks. Sufficient copper went into solution from alloys A, E, and F to exceed the copper-threshold value of even the less susceptible milks.

*Ascorbic acid oxidation.* How temperature and the length of time of exposure of the copper-nickel alloys to milk affect the rate of ascorbic acid oxidation is shown in figure 1. The milk exposed to alloy D for 1½ hours showed a very slight oxidized flavor after 3 days, whereas that exposed to G had a very strong oxidized flavor. The other milks did not show this defect.

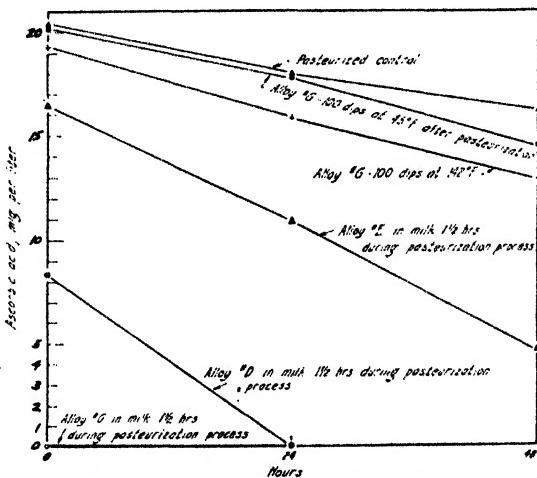


FIG. 1. Influence of temperature and time of exposure of copper-nickel alloys on the rate of oxidation of ascorbic acid in milk.

To arrive at an approximation of the amount of copper going into solution when the alloys were exposed to milk for brief periods of time, 0.1, 0.01, and 0.001 p.p.m. of cupric ion were added to other portions of the same milk into which alloys D and G were dipped 100 times at 142° F. The ascorbic-acid contents of the milk were determined immediately after treatment and after 2 and 4 days' storage at 40° F (figure 2). The milks were also scored for flavor. The raw and pasteurized samples to which 0.1 p.p.m. cupric ion were added had a very slight oxidized flavor on the fourth day, whereas the others were not influenced.

Strips of pure nickel, zinc, and lead measuring 1 inch by 2 inches were also agitated in milk during the pasteurization process. The rate of oxidation of ascorbic acid was not influenced by this amount of exposure to these metals, nor was oxidized flavor detected in any of the milks so treated.

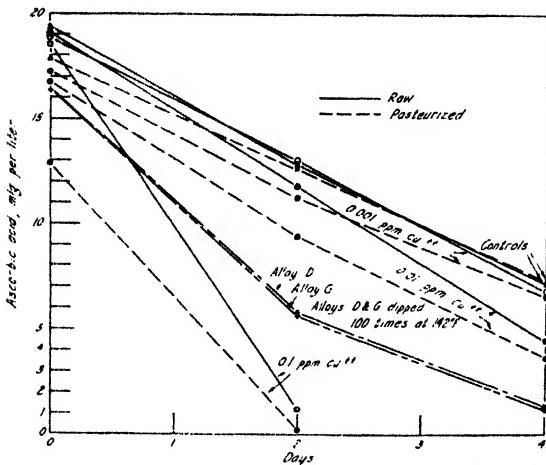


FIG. 2. Influence of cupric ion concentration on the rate of oxidation of ascorbic acid in milk.

#### DISCUSSION OF RESULTS

As is indicated by the variations in the corrosion rates of cast copper-nickel alloys and their influence on the flavor of milk, one must test each alloy experimentally in order to evaluate the desirability of its use in fabricating milk-plant equipment. A study of table 3 reveals that alloys A, E, F, and G have high corrosion rates in milk as compared with alloys B, C, and D. According to table 1, alloys A, F, and G are similar in composition in that all contain 31 per cent nickel and no tin or zinc. Alloy E, however, has a composition more nearly like that of B, C, and D, since it contains 20 per cent nickel and also tin and zinc. By analogy one would expect alloy E to have a corrosion rate more nearly like C than G. The effect on milk flavor (table 3), however, shows that E caused oxidized flavor to develop only in the more "susceptible" milks, as did B, C, and D. Figure 1 shows also that alloy E had even less effect on ascorbic acid oxidation than did D. According to these results, copper was less soluble in alloy E than in G despite their similar corrosion rates. The conclusion is that the solubility of the elements in these alloys is a complex function of their compositions, their alloy structures, or a combination of these.

When the alloys were in contact with the milks during the entire pasteurization process, alloys B, C, and D were most satisfactory as judged from the rate of corrosion and the influence on milk flavor. Alloys B and C are reported to be very difficult to machine, and the cost of the added tin over that contained in D would not be justified. Alloy D is considered the most satisfactory for commercial use.

A brief exposure of any of the alloys to milk such as would result from passing milk through fittings in a stainless-steel pipeline would not be ex-

pected to permit sufficient copper to go into solution to cause oxidized flavor to develop in mixed milks. The evidence for this is the effect on ascorbic acid destruction after the alloys were dipped 100 times in the milks at 45° and at 142° F. Judging, however, from the rates of corrosion for a longer exposure period, from the appearance of the metals after exposure to milk, and from their influence on the flavor, alloy D is the most satisfactory of the alloys studied.

The flux used in preparing the alloy at the foundry is said to affect the corrosion resistance. It is used in the molten metal to serve as a deoxidizer to remove sulfur and oxygen. The flux is finally removed from the molten alloy as slag. For the copper-nickel alloys, pure magnesium in stick form has been recommended at the foundry in which the samples studied were prepared.

#### SUMMARY AND CONCLUSIONS

Several copper-nickel alloys have been studied to determine the rates of corrosion in milk, together with the effect upon milk flavor and upon ascorbic acid destruction. The following conclusions have been drawn:

1. Less copper went into solution from the alloys containing tin and zinc, so that the flavor of the milk was less influenced than with alloys in which these elements were absent.
2. In general the rates of corrosion in milk were lower with alloys containing tin and zinc. The influence on ascorbic acid destruction, however, was found to be a more reliable index of the probable effect of the alloys on milk flavor. The effect on ascorbic acid destruction is a specific test for copper in solution: whereas the loss in weight does not necessarily indicate the relative amount of copper going into solution.
3. Nickel, lead, and zinc did not influence the oxidation of ascorbic acid and did not cause oxidized flavor in the milks pasteurized in contact with pure strips of these elements.

#### ACKNOWLEDGMENTS

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# WATER SORPTION BY DRY MILK SOLIDS. III. A COMPARISON OF RESULTS OBTAINED BY THE CRYOSCOPIC, VAPOR PRESSURE, AND VOLUME CONTRACTION METHODS

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The papers of the present series (1, 2, 3) have reported investigations on the effect of various treatments on the water-solids relationships in dry milk. These studies have been made using the volume contraction method as previously described. Other investigators have reported data on particular phases of the subject using the cryoscopic method and the vapor pressure method. It is of interest to compare results obtained by each of these methods on the same samples and to consider possible reasons for any disagreement among them.

## REVIEW OF LITERATURE

The cryoscopic method for the determination of "bound" water was devised by Newton and Gortner (4). The principle of the method depends upon the use of a dehydrating agent to take up the "free" water and then determining the freezing point of this solution of dehydrating agent and free water. When part of the total water present is "bound," a subnormal lowering of the freezing point results and the percentage of water not removed by the dehydrating agent can be readily calculated. In actual practice one mole of sucrose per liter of total water in the system is commonly used, although Briggs (5) has found that ethyl alcohol is more satisfactory in certain systems.

This method has had extensive application to biological materials and has been used to study the "bound" water content of dairy products. Pyenson and Dahle (6) have investigated the ability of dried skimmilk, prepared by the vacuum drum process to "bind" water. Their results showed that the dry material "binds" water to the extent of 60.1 per cent of its dry weight, when freshly prepared and approximately 40 per cent when four to eight weeks old.

The vapor pressure method depends upon the lesser fugacity of water in the sorbed state than in the free state. Sorbed water is not free to vaporize to the same extent as free water, and consequently does not exert its normal pressure. The relative vapor pressure is related to the degree of sorption as expressed by the water content of the system.

The vapor pressure method gained attention through the classic work of van Bemmelen (7) on silica gels. It has since been used in many fields of investigation. Supplee (8) was the first to show the equilibrium relationship between water vapor and water sorbed by milk powder. His results

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show that milk powder will take up about 14 per cent of its weight of water at 80 per cent relative humidity. Recently, Davis (9), using the same technique found that the moisture content of dried skim milk was about 17-18 per cent when in equilibrium with an atmosphere of 80 per cent relative humidity.

The volume contraction method employs the increase in density that accompanies sorption to measure the degree of sorption. Because the density of the system increases when sorption occurs there is a corresponding decrease in volume which can readily be determined in a dilatometer at constant temperature. The method has been used by various investigators (10, 11, 12, 13, 14) to study different materials but not previously for dry milk solids.

Several comparisons have been made of the different methods for determining the sorbed water-free water equilibrium. Sayre (15) compared the cryoscopic, calorimetric, and dilatometric (based on the freezing expansion coefficient of water) methods for gum arabic solutions and obtained reasonably good agreement for the averages, but he concluded that the cryoscopic method was the least reliable of the three. He pointed out that these methods yield results only at the freezing point. It has been generally recognized that the vapor pressure method does not yield values as great as the cryoscopic technique. Briggs (5, 16) has compared the two methods as has Grollman (17). Grollman, particularly, has criticised the usual equation used for calculations with the cryoscopic method as not being applicable when other solutes are present.

#### EXPERIMENTAL PROCEDURE

The cryoscopic technique was carried out as recommended by Newton and Gortner (4), measuring the freezing point depression caused by dissolving a mole of sucrose in a liter of water containing 10 per cent of dry milk solids which had been in solution 24 hours. The apparatus used was a Hortvet cryoscope and a previously standardized Beckman thermometer. The usual precautions with respect to super cooling were observed.

Aqueous vapor pressures of the samples at definite water contents were measured with a modified Regnault dew-point hygrometer. The apparatus consists of a 250 ml. Erlenmyer flask containing the sample having a known water content. Suspended within the flask through a tightly fitting stopper is a metal tube with a highly polished metal mirror soldered to its side. This apparatus is maintained at constant temperature, in this case at 20° C., until equilibrium is established between the sample and the atmosphere within the flask. Then ethyl ether or other suitable refrigerant is poured into the metal tube, a thermometer and an aspirator tube inserted and air bubbled through the ether with an atomizer bulb until fog appears on the mirror; the temperature is noted at this time and again when the mirror clears. The average of the two is considered the dew point of the atmos-

phere in equilibrium with the sample. Vapor-pressure-temperature tables give the vapor pressure in millimeters of mercury at saturation at various temperatures. With experience and care it is possible to observe fogging and clearing of the mirror within about a 0.2° C. interval. Comparison of this procedure with that outlined by Wilson (18) using sulphuric acid solutions to control humidity showed them to give similar results up to about 80 per cent relative humidity; above this humidity neither is satisfactory because of the necessity for extremely sensitive temperature control and the length of time necessary for equilibrium to be established. Supplee (8) and Davis (9) have used sulphuric acid solutions to control humidity.

Volume contraction was measured as previously described (1, 2, 3) at 20° C.

The samples studied consisted of eight different lots of dry milk solids, four prepared by the atmospheric roll process and four by the spray process from skimmilk which had been preheated at 71.1° C. (160° F.), 76.6° C. (170° F.), 82.2° C. (180° F.), and 93.3° C. (200° F.) for 30 minutes for each process.

#### RESULTS

The results of these studies have been calculated and are expressed as weight of water sorbed  
 $\frac{\text{weight of water sorbed}}{\text{weight of solids}} \times 100$ . Those obtained by volume contraction were found by extrapolation to the axes of logarithmic curves for volume contraction and degree of sorption and measuring the intercepts as explained previously (3). The cryoscopic values were calculated from the following equation.

$$\text{Grams bound water per 100 grams water in system} = \frac{\Delta_x \times 89.2}{\Delta_s}$$

Where  $\Delta$  = Freezing point of sample in solution

$\Delta_a$  = Freezing point after the addition of 32.224 gm. sucrose per 100 gm. water

$$\Delta_s = \Delta - \Delta_a$$

$$\Delta_x = \Delta_s - 2.085$$

89.2 = A constant to correct for the hexahydrate formation of sucrose

2.085 = Theoretical freezing point depression caused by sucrose.

The "bound" water corresponding to the degree of sorption is the  
 $\frac{\text{grams 'bound' water}}{\text{grams of solids}} \times 100$ .

The values for the vapor pressure studies were taken from degree of sorption—vapor pressure curves for the different samples at 80 per cent relative humidity at 20° C. These curves are shown in figure 1.

The shape of the curves makes it apparent that each represents two discontinuous functions. Supplee's results (8) show the same general form,

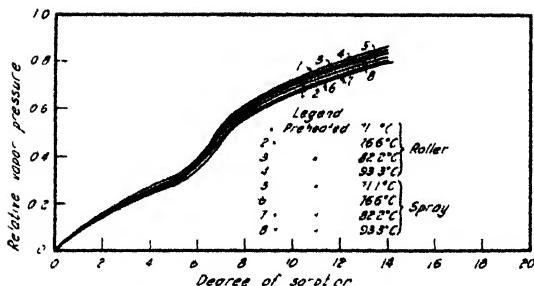


FIG. 1. The relation between degree of sorption and relative vapor pressure in dry milk solids.

while Lampitt and Bushill (19) report that spray-dried milk powder exhibits this feature but atmospheric roll dried milk does not. The shape of the curves results from the fact that the lactose is in the non-crystalline state in the dry material; it takes up water rapidly until sufficiently fluid for orientation of the sugar molecules in a crystalline pattern; then the excess water is free to vaporize and is given up. If the top portion of the curve, which represents water sorption by the proteins, is extrapolated to the point of origin a smooth curve typical of this type of material results.

It will be noted that the curves for all samples are very close together at low water contents and tend to become farther apart as they approach the saturation level.

The comparison of the results by the three methods is shown in table 1.

TABLE 1  
Comparison of results by cryoscopic, vapor pressure, and volume contraction methods for determining the degree of water sorption by dry milk solids

Sample preheating temp. (30 min.)	Degree of sorption		
	Cryoscopic method	Vapor pressure method at 80% rel. humidity	Volume contraction method
° C.	Per cent	Per cent	Per cent
71.1	24.6	12.0	15.1
76.6	26.9	13.3	18.5
82.2	24.9	12.4	15.8
93.3	25.6	12.4	16.5
71.1	42.6	12.7	29.5
76.6	53.8	14.2	36.3
82.2	46.7	13.9	23.9
93.3	44.7	12.8	23.9

Particular attention is called to the fact that the vapor pressure values were taken at 80 per cent relative humidity and so do not indicate the maximum extent to which sorption may occur when exposed to atmospheres saturated with water vapor. The values by the other methods represent maxima for each method.

It will be observed that there is fair qualitative agreement, but that the methods differ in the magnitude of the results. The cryoscopic method gives values greater than either of the others and it seems probable that the vapor pressure method will yield the smallest values although it is difficult to predict the ultimate amount of water sorption that might be obtained if measurements could be made accurately at the saturation level. In all cases the samples preheated at 76.6° C. (170° F.) for 30 minutes showed the greatest water-holding capacity and those prepared by the spray process were superior in this respect to the ones prepared on the atmospheric roll.

#### DISCUSSION

It is apparent from the qualitative agreement of the values reported that the methods measure either the same phenomenon or a closely related one. Some possible reasons for the lack of quantitative agreement need to be considered. Gortner (20) has expressed the opinion that the cryoscopic technique may be expected to yield minimal values. He points out that the dissolving of sucrose and subsequent freezing might be expected to shift the "bound" water  $\rightleftharpoons$  "free" water equilibrium to the right with the result that the values obtained at the freezing point would be lower than those obtained at higher temperatures. Considering the phenomenon from another approach, however, it appears that the equilibrium might well be shifted to the left. The sorption of water is an exothermic reaction and should increase as the temperature is lowered. Data obtained in this laboratory (21) from vapor pressure measurements indicates that dry milk solids will sorb 10.3 gm. water per 100 gm. solids at 20° C. and 14.3 gm. at 10° C. with relative humidity of 70 per cent. The difference tends to increase at higher humidities. Svedberg's data (12) show the same temperature effect. In carrying out the cryoscopic technique, even though the material is allowed to come to equilibrium at 20° C., the temperature must be lowered to the freezing point with a thermal disturbance of the equilibrium. It is perhaps significant that the equilibrium is not disturbed in either the vapor pressure or the volume contraction methods.

It should be mentioned that the vapor pressure method is the only one of the three capable of thermodynamic interpretation involving the calculation of the entropy and free energy changes. This relies on the assumption that the reaction is reversible, which is not strictly true because of the hysteresis effect; and also on the assumption that the system is homogeneous, which again is not true for dry milk solids. For these reasons the thermodynamic calculations are not given.

It is the belief of the author that the results obtained by the volume contraction method are more nearly a correct measure of the degree of sorption than those obtained by the other methods. The volume contraction is a static method and nothing is done to disturb the equilibrium as is the case with the cryoscopic method. Also, the volume contraction method can be

used to measure the degree of sorption of the dry material in direct contact with liquid water, a condition not possible by the vapor pressure method.

#### SUMMARY

1. Comparisons are given for the degree of sorption of dry milk solids as determined by the cryoscopic, the vapor pressure, and the volume contraction methods.

2. It is shown that there is qualitative agreement among the methods for differently processed samples, but the values differ in magnitude depending upon the method used.

3. The cryoscopic results for each sample are greater than those by the other methods, and the results by the vapor pressure method at 80 per cent relative humidity are the smallest.

4. Some possible reasons for the differences are discussed.

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# THE FINAL SOLUBILITY OF d-GALACTOSE IN WATER

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In the course of some research in this laboratory on sugars, information on the solubility of d-galactose in water was required. The only value found in the literature is that of Dehn (1), who gives the solubility of galactose as 68.30 grams per 100 grams of water at 20–25° C. He does not state whether he used d-galactose nor whether his value refers to a solution in which  $\alpha$ - $\beta$  equilibrium had been attained.

The d-galactose used was a high-grade Pfanstiehl product. Its specific rotation was obtained and was for a 10 per cent solution by volume  $[\alpha]_D^{20} = +80.4^\circ$ . Bacteriological tests showed the absence of glucose and lactose.<sup>1</sup> Thus a satisfactory degree of purity was indicated.

Saturated galactose solutions were prepared in two ways, the first by shaking the sugar with water at room temperature, and the second by heating the solution to 40° C. Thus equilibrium was approached from both an undersaturated and a supersaturated condition. Excess crystals were present, of course, in each case. The bottles were suspended in a thermostat kept at 25° C.  $\pm .02$ . After the solution stood for several days to permit equilibrium among isomeric forms to become established, 5 cc. samples were pipetted from each bottle, run into tared evaporating dishes and weighed. After being heated in a water-jacketed vacuum oven for several days, the dishes were weighed, and the operations repeated until constant weights were obtained. The values for each method of approach to equilibrium were in satisfactory agreement. The mean of these values gave the solubility at 25° C. as 32.09 grams per 100 grams solution. This is equivalent to a solubility of 47.25 grams of galactose in 100 grams of water.

## REFERENCE

- (1) DEHN, W. M. Comparative solubilities in water, in pyridine and in aqueous pyridine. *J. Am. Chem. Soc.*, **39**: 1399–1404. 1917.

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\* Deceased Sept. 12, 1939. Any communications regarding this paper should be addressed to E. O. Whittier, of the Bureau of Dairy Industry, who directed the investigation.

<sup>1</sup> These tests were carried out by Dr. H. R. Curren of these laboratories, to whom thanks are due.



## A METHOD FOR CALCULATING THE BAUMÉ READING OF CONDENSED ICE CREAM MIXES\*

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The manufacture of ice cream mixes in the vacuum pan has created a need for a method for the accurate forecasting of the Baumé hydrometer reading of these mixes, in order to prevent over or under condensation, and to provide a guide for indicating when the mix is ready to draw. This Baumé reading has been and is still largely determined by taking samples at varying degrees of condensation until the composition of the mix has reached the point desired. Subsequent mixes are then drawn at this predetermined reading. Apparently it should be possible to predict the proper hydrometer readings by paper analysis for mixes of given composition, provided the specific gravities of the ingredients making up the mix are known. When these methods of calculation are checked against actual analyses, however, the relationship shows considerable inaccuracy, sufficiently so as to render their commercial usage impractical.

Sharp and Hart (1) in mentioning 36 formulas for calculation of the relationship between solids and fat content of milk state an important observation, "a large part of this lack of agreement and reproducibility is due to one factor which has never been limited adequately, namely, the lag in the change in the physical state of the fat as the temperature is adjusted to that at which the specific gravity is determined." The difficulties of ascertaining specific gravity mentioned by Sharp and Hart are explained in part by Leighton, Leviton, and Williams (2, 3, 4) in their work on apparent and basic viscosity. These phenomena are variously attributed to fat clumping, specific heat, agitation, acidity, hydration, homogenization, pasteurization, forewarming temperatures, and electrical charges carried.

The Laboratory Manual of the I.A.M.D. (5) carries a table listing the Baumé readings for four mixes of varying compositions taken at five temperatures, varying from 60° F. to 140° F. Sommer (6) gave the following formula for predicting with fair accuracy the specific gravity of a mix being condensed :

Specific gravity at 60° F. =

$$\frac{100}{\frac{\% \text{ Fat}}{.93} + \frac{\% \text{ Sugar, Serum Solids, and Gelatin}}{1.58} + \frac{\% \text{ Water}}{}}$$

In the same text the specific gravity of milk-solids-not-fat was quoted as 1.5847, of sugar as 1.58, of butterfat as .93, and that of gelatin assumed to be

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1.58. The temperature at which each determination was made was 60° F. The values given by Sommer are those, he states, of Fleischman.

#### EXPERIMENTAL

##### *Estimation of Specific Gravity*

Without the interference of such factors as viscosity, surface tension, or specific heat of the various ingredients, the specific gravity of any desired composition ice cream mix could be predicted before condensing the mix, if the specific gravity of each ingredient was known. To illustrate, specific gravity of the following mix could be calculated in this manner:

12.	pounds butterfat	$\times$	density	= fat density factor
10.	" S.N.F.	$\times$	"	= S.N.F. density factor
15.	" sugar	$\times$	"	= sugar density factor
0.4	" gelatin	$\times$	"	= gelatin density factor
62.6	" water	$\times$	"	= water density factor
<hr/>				
100.0 pounds				sum of density factors

Predicted density = sum of density factors  $\div$  100. Since, specific gravity =

$\frac{145}{145 - {}^{\circ}\text{Baum\'e}}$ , or  ${}^{\circ}\text{Baum\'e} = 145 - \frac{145}{\text{specific gravity}}$ , such predicted density could be readily converted into degrees Baumé. Application of the above to practical conditions soon proved the fallacy of the reasoning. The specific gravity values given by Fleischman were determined at 60° F., whereas Baumé readings of mixes are usually made at 125° F. The object in this study has been primarily the development of a formula for accurately predicting the specific gravity and Baumé reading of ice cream mixes condensed to a predetermined content. This made necessary determinations of densities of the commoner ingredients used in ice cream, actual condensation of mixes both in laboratory and commercial size vacuum pans, temperature effect and temperature correction on Baumé readings, surface tension, and apparent and basic viscosity values for pan condensed mixes.

##### *Condensations in the Laboratory Pan*

A small experimental laboratory type vacuum pan was used for the purpose of preliminary work and with the thought of applying the findings to the operation of the larger factory size pan. It was possible to produce five pounds of finished mix with this apparatus. Four basic mixes were used. These covered in range, so far as commercial practice is concerned, the mixes commonly made in the vacuum pan.

##### *Condensations in the Commercial Size Pan*

Mixes made up in the 42 inch vacuum pan were calculated by the normal equation method and were of the varying compositions shown in table 1. The liquid ingredients were heated in the hot well to 95° F., the sugar and

TABLE I  
*Composition of basic mixes used in experiment*

Mix number	Fat	Solids-not-fat	Sugar	Gelatin
	per cent	per cent	per cent	per cent
1 . . . . .	8	11	14	0.4
2 . . . . .	8	11	15	0.4
3 . . . . .	8	11	16	0.4
4 . . . . .	10	10.5	14	0.4
5 . . . . .	10	10.5	15	0.4
6 . . . . .	10	10.5	16	0.4
7 . . . . .	12	10	14	0.4
8 . . . . .	12	10	15	0.4
9 . . . . .	12	10	16	0.4
10 . . . . .	14	9	14	0.4
11 . . . . .	14	9	15	0.4
12 . . . . .	14	9	16	0.4

gelatin added, the mix further heated to 155–160° F. with live steam, drawn into the pan and condensed to the hydrometer readings indicated by the results obtained through the trials with the laboratory size pan. About one hour was required to condense a 1000-pound mix and all mixes were 1000 or 1235 pounds in size. If condensed too far the mixes were standardized with water to desired weight. Samples for viscosity and surface tension determinations were taken after homogenization at 2300 pounds pressure. The mix was then cooled immediately, and tested in the manner described in the preliminary study. Baumé readings were made within an hour after the mixes were drawn. In making these the sample was heated to 155° F., and tested at 5° F. intervals from 155° F. to 60° F. Unless the sample was stirred occasionally in the hydrometer cylinder there was a tendency for it to oil off. Again, as in the preliminary work, pycnometer determinations for density at 70° F. were made for comparison with density determinations at 70° F., the latter being calculated by conversion to density of Baumé hydrometer determinations. For restandardization of mixes Mojonnier tests for fat and total solids were used.

#### *Density Determinations of Ice Cream Ingredients*

In an effort to utilize the simple calculation described under "Estimation of Specific Gravity" it seemed desirable to reestimate the specific gravity of the more common ingredients of ice cream and to make this estimation by what might be regarded as an unorthodox method, that of determining the specific gravity of the ingredient in solution or suspension. The specific gravity of each constituent could then be converted to Baumé hydrometer reading using the formula,  ${}^{\circ}\text{Baumé} = 145 - \frac{145}{\text{specific gravity}}$ . Since, in practice, it was learned that the use of the method of calculation mentioned gave inaccurate results, it was hoped that the effect of factors such as condi-

tion of fat, viscosity, and related factors causing the inaccuracy could be compensated for by the determination of a factor that might be used in connection with the calculation.

To make the term specific gravity synonymous with relative density, all weights were determined on an equal arm balance. If the materials in a mix do not expand when heated, density will remain the same. Liquid ice cream mix expands when heated, but so far as could be measured by ordinary means, no solid in the mix, except butterfat, expands under the influence of heat. This factor introduces a slight error in the calculations which follow. The Baumé hydrometer is calibrated to compensate for the approximate errors due to temperature variations, and this correction is probably sufficient for practical purposes. Since coefficients of expansion vary greatly for different liquids, the correction cannot be strictly accurate.

Baumé readings were made of all mixes condensed and the readings converted into density values using the formula previously given. This calculated density was then checked by a density value determined by the pycnometer method, using the mix and water at identical temperatures.

The method adopted for specific gravity determination would seem to eliminate objections to the capsule and displacement methods and to take into consideration the insoluble, colloidally soluble, and wholly soluble constituents of the mix. That porosity of water cannot provide sufficient space for sucrose is shown by the fact that when added to a measured volume of water in quantities less than saturation the volume of the mixture increases.

The method of density determination consisted in the use of a 100 ml. volumetric flask the neck of which was graduated by 0.1 ml. from 100 to 110 ml. Ten grams of skimmilk powder, previously tested for water and fat content, were weighed into the flask, and 100 ml. of boiled distilled water added at 68° F. The mixture was shaken, allowed to stand until all foam had disappeared, and volume determinations made at 5° F. intervals from the temperatures of 60° F. to 155° F. The purpose of this was to eliminate necessity of correction for expansion of glass. Several duplicate determinations were made, as well as determinations using 12 and 14 grams of skimmilk powder per 100 grams of water. The increase in the volume of water in the flask was recorded as the volume of the particular weight of skimmilk powder added.

The above determination was checked for accuracy of volume at different temperatures by calculation of density thru the use of a 25 ml. pycnometer. Weighings were made into the pycnometer at room temperature, the filled pycnometer heated to the desired temperature, dried, reweighed, and the density of its mixture content calculated by determination of the pycnometer volume at different temperatures and dividing this volume into the pycnometer weight of the mixture. By determining volume of water and mixture at the different temperatures used, no correction for glass expan-

sion was necessary. The density of the mixture was taken as the weight of all water present plus weight of dry powder divided by the volume of all water present plus the volume of dry powder. The density of the solids-not-fat was taken as the weight of dry powder divided by the volume of dry powder. Although in the same condition as when weighed into the ice cream mix the density values are not strictly accurate, due to water of hydration. A second factor causing slight inaccuracy was the fat content of the skimmilk powder, amounting to 0.1 per cent. This, calculated as water, caused a difference of less than 0.0001 in the density of solids-not-fat.

The values for density of water at varying temperatures given in Lange's Handbook of Chemistry (7) and for the density of butterfat as given by Bailey (8) were used for this work.

### *Predicted Density Determinations*

Once the densities of the various ice cream constituents were available an attempt was made to use the values for prediction of correct Baumé readings at which to draw mixes from the vacuum pan. This was done by comparing at the same temperature predicted Baumé, as calculated from density, with the Baumé reading of a mix previously made of known composition. If the two were not the same, the factor by which the predicted density should be multiplied to give a correct reading, was determined. Should this be possible it would indicate existence of a straight line relationship between the additive density of a mix and its measured density. For this reason additive densities were calculated to the fourth decimal place and the Baumé hydrometer read as nearly as possible to its smallest division, 0.1°.

## RESULTS

### *Condensation in Laboratory and Commercial Pans*

*Physical state of fat, effect on Baumé reading.* When mixes stand or cool the increase in viscosity will cause the hydrometer reading to vary, due not only to viscosity but probably due also to mechanical resistance offered by solidified and crystallized butterfat.

TABLE 2  
*Average effect of state of fat on Baumé reading of ice cream mixes*

Composition of mix	Baumé reading with fat in liquid state	Baumé reading with fat in solid state
8: 11 : 14: 0.4	13.10	13.25
8: 11 : 15: 0.4	13.35	13.50
8: 11 : 16: 0.4	13.75	13.90
10: 10.5: 14: 0.4	12.30	12.50
10: 10.5: 14: 0.4	12.80	12.95
10: 10.5: 14: 0.4	13.30	13.45
12: 10 : 14: 0.4	11.80	12.00
14: 9 : 14: 0.4	11.20	11.40

The samples above were cooled to 60° F., held four hours at 40° F., warmed to 60° F., and Baumé determinations made. Duplicates of these samples were cooled to 60° F., held four hours at 40° F., heated to 155° F., cooled to 60° F. and tested for density. These duplicates, it will be noted from the second column of the table, gave lower readings, doubtlessly, because the fat was still in an uncryallized state. The results would indicate the necessity of treating cold samples, as indicated by the treatment given the duplicates, to secure a correct Baumé reading.

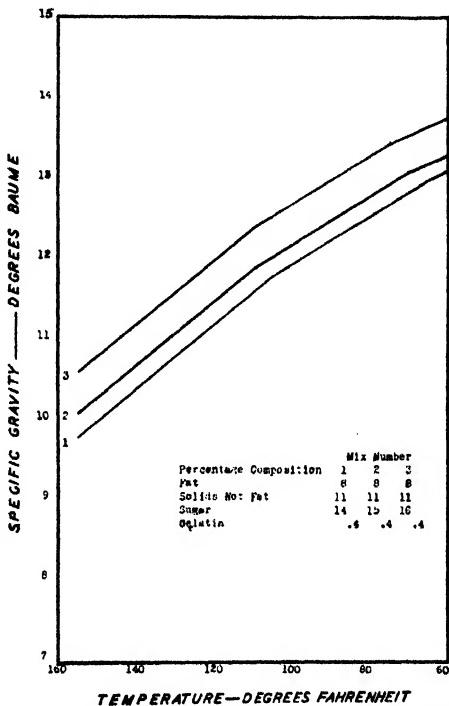


FIG. 1. Relation between temperature and Baumé readings of different composition ice cream mixes.

*Temperature effect on Baumé readings.* The Baumé readings were made from 155° F. to 60° F. at 5° F. intervals in the hope of obtaining an ideal temperature at which to take readings.

Within the range of 110° F. to 155° F., each 5° F. interval caused a change of 0.2° in the Baumé reading; from 100° F. to 70° F. the change in Baumé was 0.15° for each 5° F. change; and for 60° F. to 65° F., in most cases, 0.1° Baumé change for the 5° F. interval. Lack of uniformity in these changes per 5° F. interval may be due to the changes beginning to take place in the fat globule structure: the change takes place at 110° F.

and this point is fairly close to the solidifying point of butterfat. The time required for butterfat to crystallize would tend, however, to weaken such a theory. It is more likely due to viscosity changes. Inspection of figures 1 to 4 shows composition of mix to have little if any effect.

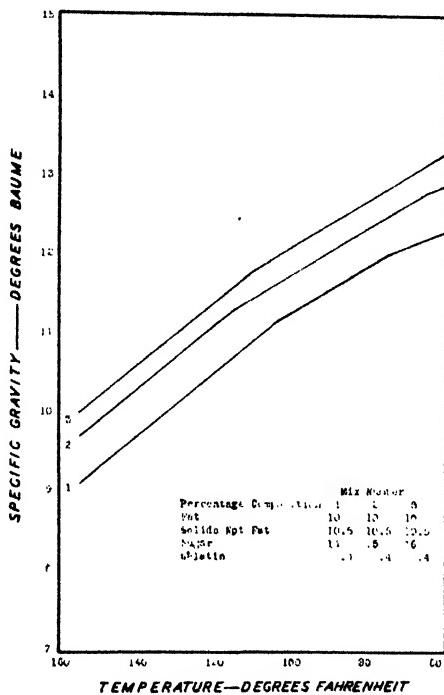


FIG. 2. Relation between temperature and Baumé readings of different composition ice cream mixes.

*Baumé and pycnometer density determinations compared.* In the following table densities of mixes are given as determined by pycnometer and hydrometer. Each determination given represents the average for each group, composed of at least two, and, in most cases three, individual mixes. Temperature corrections were made to 70° F. Baumé readings were converted to density. Since the pycnometer was calibrated to be used at 68° F. the Baumé hydrometer was corrected to 70° F. to make the results comparable. Compositions were determined by the Mojonnier method.

The above table of results possesses value chiefly as a check on the accuracy of the hydrometer reading, and on the degree of precision with which it may be read. On the average the Baumé converted readings varied 0.0011 from the pycnometer values. This is slightly more than 0.1° Baumé. Nearly one-half of the readings were more than 0.1° Baumé

greater or less than the pycnometer determination. The source of error, therefore, appears to be with hydrometer accuracy and with the operator. The greatest source of mechanical error appears to be with the adjustment of temperature. Since it seems to be possible to read the hydrometer no more closely than  $0.1^{\circ}$ , and since this variation causes a density variation

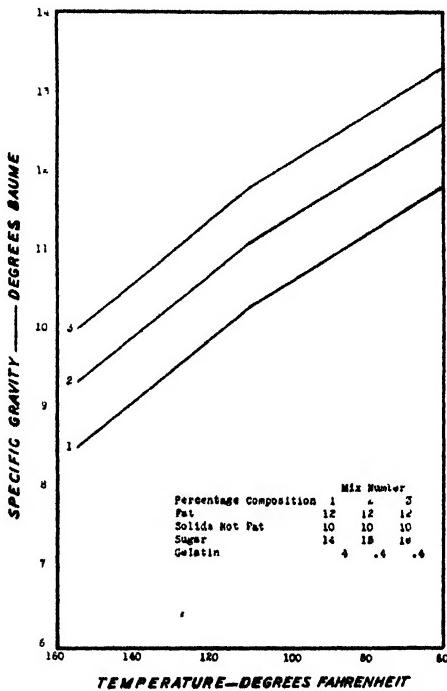


FIG. 3. Relation between temperature and Baumé readings of different composition ice cream mixes.

of 0.0008, it would seem advisable that only the best grade of rechecked hydrometers, having easily read graduations, be used.

*Homogenization effect on viscosity and surface tension.* Viscosity and surface tension will undoubtedly affect density as determined by hydrometer and any processing of the mix which greatly affects viscosity and surface tension will cause readings of one mix so treated to appear at variance with an identical mix processed normally. Unfortunately no mixes in this series were made with butter as a source of fat and the results apply only to mixes carrying fat as it occurs in milk and cream. Probably a butter mix would show less viscosity due to dispersion of fat. Viscosity was determined by the Mojonnier-Doolittle and MacMichael viscosimeters; surface tension with a du Nuoy tensiometer.

Unhomogenized mixes made in the laboratory size pan developed con-

TABLE 3  
*Average densities at 70° F. of ice cream mixes calculated by Baumé  
and pycnometer methods*

Mix				Density		
Composition Percentage				Baumé	Pycnometer	Difference
Fat	Solids-not-fat	Sugar	Gelatin			
8	11	14	0.4	1.0972	1.0980	0.0008
8	11	15	0.4	1.1009	1.1015	0.0006
8	11	16	0.4	1.1032	1.1150	0.0018
10	10.5	14	0.4	1.0841	1.0848	0.0007
10	10.5	15	0.4	1.0939	1.0934	0.0005
10	10.5	16	0.4	1.0985	1.1003	0.0018
12	10	14	0.4	1.0865	1.0890	0.0005
12	10	15	0.4	1.0927	1.0935	0.0008
12	10	16	0.4	1.0985	1.1004	0.0019
14	9	14	0.4	1.0813	1.0825	0.0012
14	9	15	0.4	1.0861	1.0864	0.0003
14	9	16	0.4	1.0927	1.0923	0.0004
13.90	9.45	15	0.4	1.0856	1.0878	0.0022
13.85	9.55	15	0.4	1.0886	1.0892	0.0006
14.50	11.0	14	0.4	1.0877	1.0863	0.0014
9.9	10.2	14	0.4	1.0841	1.0862	0.0021

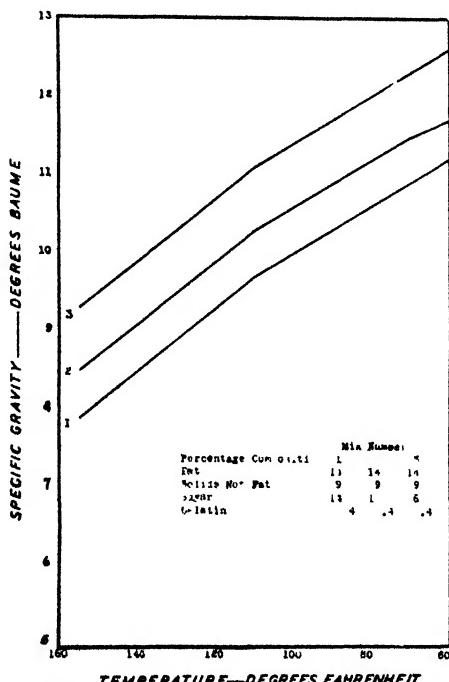


FIG. 4. Relation between temperature and Baumé readings of different composition ice cream mixes.

siderable viscosity, this amounting from one-half to nearly the same viscosity developed by identical homogenized mixes. Homogenization had little effect on viscosity of factory-size pan condensed mixes.

As a general rule increased viscosity was accompanied by a slight lowering of surface tension although the change was not sharp. With the factory-size pan homogenization caused no surface tension changes, although this probably would vary according to pressure and to condition of the homogenizing valve surfaces.

Apparent viscosity varied from twice to three and one-half times the basic viscosity. Pan condensed, homogenized mixes approximately tripled their viscosity during a 24-hour aging period. This tendency was greater with high fat and high total solids mixes. Viscosities measured by the MacMichael viscosimeter in centipoises were about three times as great as the value in degrees retardation secured by the Mojonnier-Doolittle viscosimeter.

#### *Density Determination of Ice Cream Ingredients*

*Density of solids-not-fat.* Rather than use data on coefficients of expansion of water, the 110 ml. volumetric flasks used for density determination of the solid ingredients of the mix were calibrated at several temperatures. Holding 100 ml. at 68° F., the same weight of water at 120° F. had risen in the graduated neck to a volume of 101.05 ml. Readings at other temperatures, as recorded below, were secured similarly.

TABLE 4

*Volume readings secured in calibration at varying temperatures of 110 ml. graduated volumetric flasks using boiled, distilled water*

Temperature degrees Fahr.	Volume in ml.	
	Flask 1	Flask 2
155 .....	102.05	102.05
150 .....	101.90	101.90
145 .....	101.70	101.70
140 .....	101.60	101.60
135 .....	101.40	101.40
130 .....	101.30	101.30
125 .....	101.20	101.20
120 .....	101.05	101.05
68 .....	100.00	100.00
60 .....	99.90	99.90

Increase in temperature and volume of water did not show a straight line relationship. In general the increase in volume was about 0.15 ml. per 5° F. increase in temperature. The values given in table 4 were used for determining the volume per unit of weight of skimmilk powder, gelatin, and sugar in the following manner. Ten grams of skimmilk powder were added to one of the flasks containing 100 ml. of water at 68° F., the temperature

raised to 130° F. and the volume of the mixture taken. This volume of mixture, less 101.3 ml., the volume of the water at 130° F., was taken as the volume of water displaced, or volume occupied by the 10 grams of skimmilk powder when present in water in its three states, solution, colloidal solution, and suspension. Volume of powder divided by weight of skimmilk powder was taken as the density of the skimmilk powder. Corrections were made for moisture and fat content. Densities of gelatin and sucrose were similarly determined.

TABLE 5  
*Volume readings of skim milk powder at various temperatures*

Temp. deg. Fahr.	Volume 100 ml. water	Volume 100 ml. water 10 grams pwd.	Volume 100 ml. water 12 grams pwd.	Volume due to		Density of S.N.F.
				10 gms. powder	12 gms. powder	
60	99.90	106.10	107.34	6.30	7.44	1.6129
68	100.00	106.20	107.44	6.30	7.44	1.6129
120	101.05	107.25	108.49	6.30	7.44	1.6129
125	101.20	107.40	108.64	6.30	7.44	1.6129
130	101.30	107.50	108.74	6.30	7.44	1.6129
135	101.40	107.60	108.84	6.30	7.44	1.6129
140	101.60	107.80	109.04	6.30	7.44	1.6129
145	101.70	107.90	109.14	6.30	7.44	1.6129
150	101.90	108.10	109.34	6.30	7.44	1.6129
155	102.05	108.25	109.49	6.30	7.44	1.6129

From table 5, it will be noted that an increase in temperature did not cause a change in volume of the skimmilk powder, using either 10 grams or 12 grams in 100 ml. water. This verified preliminary data secured when 10, 12, and 14 grams of skimmilk powder were used, results of which are not recorded here.

TABLE 6  
*Density of solids-not-fat suspensions from volume readings using 10 grams powder*

Temperature	(10 gms. powder—100 ml. water)	
	By pyrometer	By volume
60	1.0351	1.0358
68	1.0351	1.0350
120	1.0375	1.0355
125	1.0372	1.0355
130	1.0364	1.0356
135	1.0378	1.0358
140	1.0380	1.0358
145	1.0367	1.0358
150	1.0360	1.0359
155	1.0354	1.0360

$$\text{Density of SNF at any temperature} = \frac{\text{Wt. SNF}}{\text{Vol. SNF}} \text{ at any temperature.}$$

Column 2 of table 6 contains the values obtained by pycnometer weights. In each case all determinations were corrected to 60° F., using the values given in table 7, for the reason that the pycnometer was also corrected to 60° F. These were of service in checking individual determinations. The same system was followed for the values in column 3, and for density of solids-not-fat given in table 5. It was followed, also, in the calculations for densities of solids-not-fat, sugar, and gelatin. These were 1.6129, 1.6107, and 1.5384 respectively.

Density calculations gave identical values for solids-not-fat when either 10 or 12 grams of skimmilk powder were used. This is to be expected, however, since the skimmilk solids did not change volume with the variations in heat used. The density value secured, however, was from the suspension and solution of the skimmilk in water, and is literally the density due to the solids-not-fat in suspension in the water. Density of the suspension by the volume and pycnometer methods checked fairly closely.

Density of sugar was the same at all temperatures used. One and two grams of gelatin were used per 100 ml. of water in checking its density. While this is much greater than the amount used in ice cream mix this quantity was necessary for accuracy in the particular method used. As with sugar and solids-not-fat, gelatin did not increase in volume as temperature increased. Its density, therefore, was found to be uniform at all temperatures, within the range used.

*Density of butterfat and water.* The densities of butterfat and water, as given in the following table, are calculated from values given by Bailey and Lange's Handbook of Chemistry previously cited. Bailey gives the density change for butterfat as 0.00038 per degree Fahrenheit change, and the density as 0.9 at 113° F.

TABLE 7  
*Density of butterfat and water at various temperatures*

Temperature deg. Fahr.	Density of butterfat	Density of water
60	0.92014	0.99905
68	0.9016	0.99823
120	0.8974	0.98856
125	0.8955	0.98729
130	0.8936	0.98597
135	0.8917	0.98507
140	0.8898	0.98324
145	0.8879	0.98262
150	0.8860	0.98032
155	0.8841	0.97881

Density values above unity are given in the Handbook cited above and the first two columns of table 8 are copied from it. From values given in these two columns, values given in column three were calculated for use in prediction of Baumé reading. Most density readings, covering the normal

range of 1.06 to 1.11, may easily be translated into Baumé reading through the use of the table.

TABLE 8  
*Relation between density and Baumé scale for densities above unity*

Density	Baumé	Density to make 1° Baumé change
1.05	6.91	0.00752
1.06	8.21	0.00763
1.07	9.49	0.00781
1.08	10.78	0.00775
1.09	11.97	0.00840
1.10	13.18	0.00826
1.11	14.37	0.00840
1.12	15.54	0.00854
1.13	16.68	0.00877
1.14	17.81	0.00885

*Prediction of Baumé reading according to mix composition.* Inspection of table 8 will show that a change of .0008 in density is necessary to cause a change of 0.1° Baumé. The Baumé hydrometer, under practical conditions, can be read no more closely than 0.1°. It would be possible, therefore, to predict density within 0.0008 provided the hydrometer were read as closely as 0.1°. In preparing composition comparisons of actual determinations by Baumé and calculated Baumé the density values of the ingredients used were, butterfat at 113° F., 0.9; solids-not-fat, 1.613; sugar, 1.61; gelatin, 1.54; and water at 60° F., 0.99823. The densities of the second, third, and fourth were calculated as being constant through the 60–155° F. temperature range. Changes of densities for butterfat and water are given in table 7. Mixes were condensed to a predetermined composition and Baumé readings made at the temperatures specified. These readings were converted to density values. The additive densities at these varying temperatures were then calculated and divided into the corresponding density obtained from the Baumé reading. The result was the factor used for multiplying additive density at the three temperature ranges used. This value times *added density of mix ingredients* gave *faktored density* or the density nearest that obtained by actually making and testing the mix. The calculation involved merely the determination of a factor which, when multiplied by predicted density, gave a value that could be converted into the proper Baumé at which the mix was to be drawn.

Seventy-two mixes were made up to desired composition and their Baumé test read. The additive density of these mixes was calculated for the temperature range of 120–135° F. When multiplied by the factor 0.949 the average difference for the 72 mixes between predicted and determined density was 0.00175. This density variation would amount to about a 0.2° variation in Baumé, within the range specified, and, normally, all commercially condensed mixes are read within this range.

With the mix at 60° F. at which it is more viscous and the fat in a solid state, and using the factor 0.955, an average accuracy of 0.2° Baumé was obtained with the 18 readings observed. When Baumé reading was calculated from additive density the extreme variation from the observed reading was 0.7°. Of the 18 readings at 60° F. the predicted density was within a reading range of 0.1° Baumé of observed readings in 33 per cent of the readings. This number of checks, using the factor 0.955, is perhaps too few to be considered conclusive.

Using the factor 0.949 to predict the density of a mix within the temperature range of 120–135° F., 45.83 per cent of the readings were within 0.1° of the observed Baumé reading. Of the 90 readings taken, 45.55 per cent were within 0.1° Baumé of observed readings. Forty-three readings were higher than 0.0016 density allowance for 0.2° Baumé and 47 readings were below this value.

#### *Sources of Error*

The use of these factors for the temperature ranges mentioned was necessary because of the conditions which tended to change the direction of the lines showing the relationship between specific gravity and temperature (figures 1 to 4 inclusive). There may be other sources of error, also, in predicting density, most of which are directly related to accuracy in making readings and tests. Thus a density variation of 0.0008 corresponds to 0.1° Baumé change. A slight error in the Mojonnier determination for fat or total solids would easily cause 0.1° Baumé reading change. In calculating the mix, an accurate determination of fat and solids must be made rather than fat determination only, with assumption that solids-not-fat are present to the extent of nine per cent. An error in this determination may be as important as an error in the Baumé reading itself or one in predicting density. This is true especially at the higher temperature ranges where the differences between the density of water and butterfat, and those of sugar, milk-solids-not-fat, and gelatin are marked.

Losses of portions of the mix ingredients, especially solids, during condensing will alter the mix composition. When the product is standardized back these are not accounted for. The composition, therefore, is not exactly as calculated, and predicted densities from composition are not strictly correct. An error of 0.1 per cent in solids-not-fat test causes an apparent increase or decrease of 0.1 per cent water, causing a change of 0.0008 in density or 0.1° Baumé reading, *i.e.*, when the reading is made at 125° F., which is normally the temperature at which it is made, and with a mix of 8:11:14:0.4 composition. Slight changing of the amounts of those solids heavier than water would cause a corresponding change in Baumé reading.

Using specially built apparatus having greater accuracy in measurement of volume there is the possibility that additive densities at different temperatures would not give a graph in the form of a straight line. Using the

apparatus described in this study, however, the changes in volume, if any, were too small to be observed when the material was in suspension and solution in water. With volumetric apparatus reading to fractional parts of a milliliter it is believed that very exact Baumé determinations could be predicted from desired composition of mix.

#### SUMMARY

The Baumé hydrometer may be used as an accurate indicator of the composition of ice cream mixes during condensation and as a method for prediction of when to strike the batch. This work was designed to originate a method for prediction of Baumé readings for mixes of a definite finished composition, using composition as the basis for calculation. In preliminary work, homogenization of the mix was found to have no effect on Baumé reading; that, to be comparable from day to day, readings had to be taken at the same temperature; that the average mix condensed at a 24 inch vacuum boiled at 140° F., which made the temperature of 125° F. a convenient one at which to take Baumé readings; that density of all mixes studied varied 0.0016 and Baumé reading varied 0.2° for each 5° F. change in temperature within the range of 115–155° F.; that pan condensed mixes are similar in viscosity and surface tension to vat prepared mixes; and that, as a rule, great increase in viscosity resulted in slight increase in surface tension.

Twelve mixes of different compositions were made up, condensed, analyzed, restandardized to desired composition and their Baumé readings taken for use as standards. By using this standard it was found that a 1235 pound mix could be drawn to within ten pounds of its desired weight. Within the range of 60° F. to 155° F. milk solids-not-fat, sugar, and gelatin were found with increase in temperature to remain constant in volume when in suspension or solution. Densities of ice cream ingredients, determined by the methods described, were found to be as follows: milk-solids-not-fat, 1.6129; sucrose, 1.6107; and gelatin, 1.5384. Use was made of densities of water and butterfat, as given in table 7.

Additive density was calculated, as described, using the value for water and butterfat at the temperature at which the Baumé was to be read. This additive density, divided by 100, and the result multiplied by the factor, 0.949, for readings within the temperature range of 120° F. to 135° F., gave a predicted density, which when converted to Baumé give an average density accuracy within 0.2° Baumé.

To make use of these values in predicting Baumé reading for a mix the number of pounds of each component of the mix in 100 pounds is multiplied by the density of the ingredient at the temperature at which the Baumé reading is to be made. Density of milk-solids-not-fat, sugar, gelatin, and butterfat and water have been given. The density factors are added, di-

vided by 100, and multiplied by 0.949, if the Baumé reading is to be made between the optimum temperature range of 120° F. to 135° F. The density value secured is converted to Baumé by reference to table 8. This value should give correct Baumé, within 0.2°, at which to strike the mix.

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# SOME FACTORS RESPONSIBLE FOR VARIATIONS IN THE ACID NUMBERS OF THE FAT IN CREAM AND IN COMMERCIAL BUTTER<sup>1</sup>

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In a previous article it was shown that the acid numbers of the fat of commercial butter varied rather widely. No close correlation existed between the acid number of the fat and the quality of commercial unsalted butter; butter of good quality often had relatively high acid numbers, while some rancid samples had relatively low acid numbers. In order to determine some of the possible causes for the significant fat acid numbers variations observed in commercial butter a number of experimental trials were made.

## EFFECT OF THE NORMAL MIXED FLORA AND MILK LIPASE IN RAW CREAM ON THE ACID NUMBER OF THE FAT

Raw cream from several sources was used in the trials to determine the degree of hydrolysis caused by lipolytic organisms and by lipase in cream. Palmer (12) reported that 1 part of formaldehyde in 1500 parts of cream inhibited the growth of most organisms with no detrimental effect on the milk lipase.<sup>2</sup> In order to determine the effect of formaldehyde on pure cultures of some of the lipolytic organisms commonly present in raw cream, small lots of sterilized cream were inoculated with known lipolytic organisms. Formaldehyde was immediately added to the cream in concentrations ranging from 1 part in 4800 parts to 1 part in 1200 parts of cream. These lots of cream were churned after holding 6 days at 21° C. The effect of formaldehyde on the organisms was determined by their ability to grow as evidenced by increases in the acid number of the fat.

Organisms varied considerably in their tolerance for formaldehyde (table 1). *Ach. lipolyticum*, *Myc. lipolytica* and *Ps. fluorescens* grew very little in a concentration of 1 part formaldehyde to 4800 parts of cream, while *O. lactis* grew luxuriantly in all concentrations up to 1 part in 2000 parts of cream. None of the organisms showed appreciable activity in cream containing 1 part of formaldehyde in 1600 parts of cream. As a result of these trials it was assumed that any lipolysis which occurred in raw cream containing 1 part formaldehyde to 1500 parts cream was due largely to the action of

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<sup>2</sup> Recent work by R. L. Herrington and V. N. Kruckovsky has established the presence of two lipases in milk. One is inhibited completely by small amounts of formaldehyde, the other is not sensitive to moderate amounts of it. J. DAIRY SC., 22: 127-135. 1939.

milk lipase while in the cream containing no formaldehyde, the lipolysis was due to the combined action of lipase and micro-organisms.

TABLE 1  
*Resistance of certain lipolytic microorganisms to formaldehyde in cream  
(Acid number of original fat 0.6)*

Concentration of formaldehyde in cream	Acid number of butterfat after incubating cream 6 days at 21° C.			
	Cream inoculated with			
	<i>Ach. lipolyticum</i>	<i>Myc. lipolytica</i>	<i>P.s. fluorescens</i>	<i>O. lactis</i>
0	3.3	30.6	5.0	16.3
1-4800	1.0	1.3	1.5	16.4
1-3600	.9	1.8	1.4	13.3
1-2400	.9	1.2	1.2	7.9
1-2000	.9	1.1	1.1	6.7
1-1600	.9	1.2	1.0	.8
1-1400	.9	1.3	1.0	.7
1-1200	1.0	1.5	1.2	.8

Small portions of several lots of raw cream from different sources, with and without formaldehyde added, were stored for 2, 4, 6, 10 and 14 days at 5°, 13° and 21° C. The lots of cream were then churned and acid numbers of the butterfat determined.

The data shown in tables 2 and 3 reveal that in general, the lipolysis in the samples containing no formaldehyde was greatest at the lowest temperature. At 21° C. the rate of acid formation was considerably greater than at the lower temperatures which apparently tended to check the growth of some of the lipolytic organisms. Exceptions to this generalization were occasionally encountered, as for example in table 2, the cream stored at 21° C. for 14 days showed a higher fat acid number than did another lot of the same cream stored at lower temperatures. In the samples containing formaldehyde supposedly only the milk lipase was active in splitting the fat. The lipase in these samples caused hydrolysis at all temperatures studied but increases in its activity were evident as the temperatures of storage increased. The acid numbers of the fat of one group of these samples containing formaldehyde (table 2) after 14 days storage at 5°, 13° and 21° C. were 6.0, 7.5 and 13.2, respectively. Increases in the titratable acidity of the samples of cream treated with formaldehyde also occurred at all temperatures studied. From the same table it may be observed that from the original titratable acidity of 0.16 per cent, the acidity of the samples containing formaldehyde increased in 14 days at 5°, 13° and 21° C. to 0.36, 0.40 and 0.48 per cent, respectively. These increases were probably due to liberation of certain fatty acids by lipase. Increases in the acid numbers of the fat were roughly proportional to the increases in titratable acidity. The increases in acid numbers and titratable acidities were thought to be

TABLE 2

*Effect of the normal mixed flora and milk lipase in raw cream on the acid number of the fat  
(Cream separated from mixed milk of several cows)*

Days held at	Normal raw cream			Formaldehyde added (1-1500)		
	Per cent acidity	Acid number	Flavor	Per cent acidity	Acid number	Flavor
<i>5° C.</i>						
0	.16	0.9	good	0.16	0.9	good*
2	.26	6.5	bitter, rancid	.21	3.5	oxidized
4	.35	13.2	bitter, rancid	.24	3.9	oxidized
6	.51	11.1	bitter, rancid	.28	4.5	rancid
10	.60	15.2	bitter, rancid	.28	5.3	rancid
14	.69	17.1	bitter, rancid	.36	6.0	rancid
<i>13° C.</i>						
0	.16	.9	good	.16	.9	good
2	.58	7.6	rancid	.22	4.0	rancid
4	.70	8.2	rancid	.24	4.8	rancid
6	.88	7.6	rancid	.30	5.5	rancid
10	1.00	10.1	rancid	.35	6.2	rancid
14	1.14	11.5	rancid	.40	7.5	rancid
<i>27° C.</i>						
0	.16	.9	good	.16	.9	good
2	.70	6.5	good	.22	4.9	oxidized
4	.70	8.2	very sour	.28	4.8	oxidized
6	.97	9.0	rancid, cheesy	.38	7.5	rancid
10	1.03	11.5	rancid, cheesy	.40	9.5	rancid
14	1.05	22.2	rancid, cheesy	.48	13.2	rancid

\* Formaldehyde was detectable in all samples containing it; the term "good" was used to indicate the absence of a rancid flavor or odor.

due largely to the action of milk lipase on the fat since plate counts on these samples revealed relatively few organisms. Rarely did the plates show more than a few hundred organisms per milliliter. Long (10) and Collins (3) reported that organisms must be present in reasonably large numbers to cause defects and it is believed that there were too few organisms in these samples to cause the acidity increases observed. This statement agrees with the findings of Kruckovsky and Sharp (8) who showed that raw milk on standing at temperatures too low for bacterial growth, increased considerably in titratable acidity.

In some of the samples containing no formaldehyde marked increases in the acid number of the fat were noted between 10 and 14 days of storage. These sharp increases probably were caused by more rapid mold growth during this period.

The data in table 4 show that when milk from individual cows was held at 5° C., fatty decomposition occurred due to growth of organisms and action of lipase very similar to that observed in mixed herd milk.

Of the two biological agencies capable of causing lipolysis as measured by the acid number of the fat, the action of micro-organisms was of somewhat greater importance than the action of milk lipase. In the raw cream

TABLE 3

*Effect of the normal mixed flora and milk lipase in raw cream on the acid number of the fat  
(Cream separated from mixed milk of several cows)*

Days held at	Normal raw cream			Formaldehyde added (1-1500)		
	Per cent acidity	Acid number	Flavor	Per cent acidity	Acid number	Flavor
5° C.						
0	.14	0.6	good	.14	0.6	good*
2	.26	6.5	rancid	.21	3.5	rancid
4	.35	13.2	rancid	.24	3.6	rancid
6	.51	16.0	rancid	.28	4.1	rancid
10	.62	21.1	rancid	.30	5.0	rancid
14	.75	27.0	rancid	.33	6.8	rancid
13° C.						
0	.14	.6	good	.14	.6	good
2	.58	7.6	rancid	.22	4.0	rancid
4	.70	8.4	rancid	.24	4.8	rancid
6	.88	9.1	rancid	.30	5.0	rancid
10	.90	11.2	rancid	.32	6.3	rancid
14	.92	13.1	rancid	.34	7.5	rancid
21° C.						
0	.14	.6	good	.14	.6	good
2	.70	6.5	rancid	.22	4.9	rancid
4	.76	9.6	rancid	.28	5.7	rancid
6	.96	11.0	rancid	.38	6.0	rancid
10	.95	12.3	rancid	.40	7.5	rancid
14	.97	14.5	rancid	.41	9.2	rancid

\* See footnote, table 2.

in which both micro-organisms and normal milk lipase had been active the acid numbers of the fat were often 3 to 4 times greater than in the samples containing formaldehyde. This is particularly well demonstrated by the data shown in table 3. The samples held at 5° C. containing formaldehyde at 4, 6, 10 and 14 days had acid numbers of 3.6, 4.1, 5.0 and 6.8, respectively while similarly held samples of the same cream containing no formaldehyde had acid numbers of 13.2, 16.0, 21.1 and 27.0, respectively. The increases in acid numbers due to growth of micro-organisms were much greater than the increases due to milk lipase. The data presented reveal an unusual circumstance in that in every case the total lipolysis at 13° C. was less than at either 5° or 21° C. after 14 days of storage. No explanation is offered for this condition.

Davies (4) reported that certain metals tended to inhibit lipase activity in butter. In order of their inhibiting power were copper, iron, nickel, cobalt, manganese and chromium. Tin and aluminum had no effect. In the trials reported no effort was made to check or control the normal metal contamination. The results obtained may have been influenced by this factor.

Rice and Markley (13) reported that one of the causes of rancidity in dairy products is the carrying over of the enzyme into the manufactured

products. In view of the wide use of the pasteurization process for dairy products it seems doubtful that a significant carry-over of lipase would occur under ordinary factory conditions.

TABLE 4

*Effect of the normal mixed flora and milk lipase in raw cream on the acid number of the fat  
(Cream separated from milk of individual cows)*

Cow	Fresh cream		Cream after 14 days at 5° C.			
	Acid number of fat	Flavor	Normal		Formaldehyde added (1-1500)	
			Acid number	Flavor	Acid number	Flavor
1	0.5	good	4.0	rancid, sour	1.2	rancid
2	.6	good	2.6	rancid, sour	.8	rancid
3	.5	good	2.2	sour	1.9	putrid, sour
4	.5	good	3.1	fair	.9	putrid
5	.6	good	2.0	fair, rancid	1.7	rancid
6	.6	good	1.6	rancid, sour	.9	rancid
7	.7	good	5.0	rancid	3.0	sl. rancid
8	.9	good	2.2	rancid	1.2	sl. rancid
9	.7	good	2.8	rancid	1.5	old
10	.7	good	1.6	rancid	1.0	rancid

From the data presented it may be readily seen that fatty decomposition occurred in cream even when stored at low temperatures. All samples of normal raw cream contained lipolytic micro-organisms which were capable of causing hydrolysis of fat if conditions favored their growth. These results agree with the findings of Hanmer and Collins (5) who showed that lipolytic organisms were common in fresh raw milk and cream. A fat splitting enzyme was also present in all samples studied. Both of these agencies were active throughout a wide range of temperature. There was, however, considerably more hydrolysis of the fat at all temperatures in the samples containing no formaldehyde which indicates that micro-organisms were active in splitting the fat. While considerable variation may be expected in the degree of fat decomposition in cream from different sources, the importance of procuring and processing cream by the creamery soon after it is produced is emphasized.

#### EFFECT OF THE GROWTH OF BUTTER CULTURE ORGANISMS AND *L. bulgaricus* ON THE ACID NUMBER OF THE FAT OF CREAM

Most of the butter manufactured in the United States is made from gathered cream, only a comparatively small amount being made from milk separated in creameries. Gathered cream is received by creameries in some areas in a sweet condition while in others it often is excessively sour. In most of the butter producing areas the maximum acidity encountered in

cream is 0.8 to 1.0 per cent and this acid is largely the result of the growth of *S. lactis* organisms. In some sections however cream sometimes develops an acidity considerably in excess of 1.0 per cent. Such an acidity is largely the result of the growth of lactobacilli. Because of poorly organized procurement systems and lax cream grading regulations, cream often remains on farms and in cream stations for prolonged periods before delivery. This situation, coupled with high temperatures, provides conditions suitable for the growth of lactobacilli.

Plant practices often involve the ripening of cream. In this process the acidity may be increased materially before the cream is churned. Under the conditions described the acidity produced is primarily the result of the fermentation of lactose by *S. lactis* which results in the production of lactic acid. The effect of the growth of these homofermentative organisms in cream on the acid number of the fat has been investigated. In trials, portions of sweet cream were sterilized, inoculated with 1 per cent butter culture and ripened at 21° C. to varying acidities. Other lots of cream were inoculated with cultures of *L. bulgaricus* and ripened at 37° C. to acidities considerably above one per cent. The lots of cream were then cooled, churned and the acid numbers of the fat determined.

The data in table 5 shows that even though the acidities of the cream

TABLE 5

*Effect of the growth of butter culture organisms on the acid number of the fat of cream  
(Cream incubated at 21° C.)*

Trial I		Trial II		Trial III	
Per cent acidity in cream	Acid number of fat	Per cent acidity in cream	Acid number of fat	Per cent acidity in cream	Acid number of fat
0.11	0.60	0.09	0.55	0.11	0.65
.39	.65	.27	.50	.25	.60
.49	.60	.35	.50	.35	.65
.58	.60	.60	.50	.58	.65
.80	.65	.82	.55	.85	.60
.86	.60	.89	.50	.88	.65

were increased to the normal maximum of butter culture organisms, which is considerably above the normal churning acidity, the acid number of the fat was not changed appreciably. Likewise, the data in table 6 reveal that the growth of the *L. bulgaricus* failed to alter the acid number of the fat. These results indicate that growth of the common homofermentative organisms in cream is not responsible for increases in the acid number of butterfat and confirm the findings of Orla-Jensen (11) and of Laxa (9) whose data show that ordinary milk souring bacteria had no influence on the acid number of the fat.

TABLE 6

*Effect of the growth of *L. bulgaricus* on the acid number of the fat of cream  
(Cream incubated at 37° C.)*

Trial I		Trial II	
Per cent acidity in cream	Acid number of fat	Per cent acidity in cream	Acid number of fat
0.10	0.70	0.13	0.45
.88	.65	.75	.50
1.23	.65	1.30	.55
1.60	.70	1.52	.45
1.88	.75	2.02	.45

EFFECT OF GROWTH OF CERTAIN LIPOLYTIC ORGANISMS IN CREAM AND BUTTER  
ON THE ACID NUMBER OF THE FAT

Several organisms which showed definite lipolysis when grown on an agar medium containing fat emulsion were inoculated into portions of sterilized cream. After 7 days incubation at 21° C. the cream samples were churned and the acid numbers of the fat determined.

While all of the organisms showed definite lipolysis on agar plates (table 7), some of them failed to produce rancidity in cream or butter or to cause marked increases in the acid number of the fat. Some of the organisms caused increases in the acid number of the fat and yet failed to produce a typically rancid odor.

Hammer and Collins (5) reported that the highest lipolytic counts were secured on butter that was cheesy rather than rancid. Long (10) also found that certain cultures showing lipolysis on plates containing fat often failed to produce rancidity in butter. Various flavors were produced by the organisms studied, including old, acidy, roquefort, putrid, cheesy and rancid. The acid numbers on the fat of the inoculated samples after incubation ranged from 0.6 to 16.8.

Orla-Jensen (11) showed that certain organisms were able to bring about rancidity and cause high acid values on the fat. In working with pure cultures of *Ps. frugi*, Hussong (6) found that this organism was quite actively lipolytic and caused an increase in the acid number of the fat which was accompanied by a rancid flavor and odor. His work also showed that certain organisms caused marked increases in the acid number of the fat but failed to produce a rancid odor and that many lipolytic organisms are also proteolytic.

It has been shown by many workers that certain micro-organisms have the ability to hydrolyze fat when growing in cream and in butter. A series of trials were made to determine the comparative lipolytic activity of several organisms when growing in cream and in butter. Sweet cream was sterilized in an autoclave, cooled to 21° C., and inoculated with a culture of the organism under consideration. The inoculated cream was well mixed

TABLE 7

*Effect of growth of lipolytic organisms in cream on the acid number of the fat*

Cream incubated 7 days at 21° C. after inoculation	Per cent acidity in cream	Acid number of fat	Flavor of cream
<i>P. roqueforti</i>	.32	8.6	roquefort
<i>Myc. lipolytica</i>	1.57	12.4	acid, yeasty
<i>Ps. fragi</i>	.25	.6	old
<i>Ps. fluorescens</i>	.50	9.5	old, putrid
<i>Ach. lipolyticum</i>	.39	9.4	putrid, rancid
<i>Alc. lipolyticus</i>	.43	16.8	cheesy, rancid
<i>O. lactis</i>	.48	10.1	acid
Unidentified bacillus A	.30	1.8	putrid
Unidentified bacillus B	.53	3.5	putrid
Unidentified bacillus C	.32	3.4	putrid, rancid
Unidentified bacillus D	.37	1.9	roquefort, rancid
Cream before sterilization	.16	.8	good
Cream after sterilization	.13	.7	good, heated

and divided into two portions. One of these portions was churned, the butter was packed in sterile containers and placed in storage at 5°, 13° and 21° C. The other portion of the inoculated cream was carefully transferred to sterilized fruit jars and placed in storage at the same temperatures as the butter. After 4, 6, 10 and 14 days of storage, samples of cream and butter were removed from storage. The cream was churned and flavor of the butter and acid number of the fat of each sample were determined. In addition the titratable acidity of the cream was determined.

The data showing the lipolytic activity of *Ach. lipolyticum* is presented

TABLE 8

*Comparative lipolytic action of Ach. lipolyticum in cream and in butter*

Days held at	Cream			Butter	
	Per cent acidity	Flavor	Acid number	Flavor	Acid number
5° C.					
0	0.13	good	1.0	good	1.0
4	.30	good	1.3	good	1.3
6	.31	old	2.0	old	1.4
10	.32	old	2.7	rancid	1.6
14	.32	sl. rancid	3.1	sl. rancid	1.7
13° C.					
0	.13	good	1.0	good	1.0
4	.31	good	2.0	good	1.6
6	.34	old	4.3	off-flavor	1.8
10	.36	sl. rancid	4.9	rancid	2.4
14	.35	sl. rancid	5.9	rancid	3.1
21° C.					
0	.13	good	1.0	good	1.0
4	.32	old	2.8	good	1.9
6	.33	old	3.3	sl. rancid	2.7
10	.41	rancid	6.2	rancid	3.5
14	.40	rancid	7.1	rancid	4.5

in table 8. The acid numbers on both the fat of cream and of butter increased progressively throughout the 14 day period. In general the growth at all temperatures was more rapid in cream than in butter as is evidenced by the greater acid numbers on the fat of cream than on the fat of butter under the same holding conditions. The acid numbers in both cream and butter increased most rapidly at the higher temperatures, being at the end of 14 days in the cream 3.1, 5.9 and 7.1 when held at 5°, 13° and 21° C., respectively, and in the butter under the same holding conditions the acid values were 1.7, 3.1 and 4.5. This organism formed comparatively little acid at any temperature, 0.41 per cent being the maximum. In general, off-flavors were evidenced in the cream and butter after about the same period of storage at each temperature regardless of differences in acid numbers.

The results with *Myc. lipolytica* (table 9) were similar to those obtained with *Ach. lipolyticum* with the exception that greater increases in the acid numbers of the fat were observed at all temperatures and all storage periods. After 14 days the acid numbers of the fat of the cream were 34.0, 39.7 and 42.0 at 5°, 13° and 21° C., respectively, and in similarly handled butter the corresponding values were 7.0, 27.9 and 32.6.

*O. lactis* grew more luxuriantly in cream than in butter at all temperatures. This greater growth was evidenced by larger acid numbers on the fat of cream as shown in table 10. After 14 days the acid values in cream were 15.5, 19.4 and 43.0; in butter the corresponding values were 4.1, 14.8 and 38.5. This organism caused greater fatty breakdown in both cream and butter at all temperatures than did the *Ach. lipolyticum* but did not cause as much fat hydrolysis as *Myc. lipolytica* at 5° or 13° C.

The data presented substantiate the statement that the organisms studied, which included common bacterial, mold and yeast species, grew more luxuriantly in cream than they did in butter. The differences were greater at 5° than at 13° and 21° C. It is recognized that the amount of working butter receives influences the rate of growth of the organisms it contains. Since it was impossible to accurately control the degree of working, comparisons between the rate of fat breakdown in the various lots of butter containing different organisms should not be seriously considered. However since all lots of cream were very similar in fat content and other properties it seems logical that comparisons could be made of the lipolytic activity in cream of the various organisms studied.

All of the lots of experimental cream and butter became off-flavored and unmarketable after a few days of storage at all temperatures studied. Using the flavor of the butter and the acid number of the fat as criteria, it may be concluded that all of the organisms studied were extremely damaging to the quality of cream and butter. It may be further concluded that the bacterial species studied, including *Ach. lipolyticum*, *Ps. fluorescens* and *Alc. lipolyticus* (data on latter two not shown), were less damaging to cream

and butter from the standpoint of fatty decomposition than either *O. lactis* or *Myc. lipolytica*.

TABLE 9  
Comparative lipolytic action of *Myc. lipolytica* in cream and in butter

Days held at	Cream			Butter	
	Per cent acidity	Flavor	Acid number	Flavor	Acid number
<i>5° C.</i>					
0	.12	good	0.9	good	0.9
4	.16	good	1.0	good	1.8
6	.17	good	3.0	good	3.3
10	.19	rancid	11.7	sl. rancid	5.2
14	.23	v. rancid	34.0	v. rancid	7.0
<i>13° C.</i>					
0	.12	good	.9	good	.9
4	.21	old	7.4	sl. rancid	9.9
6	.27	old	13.8	rancid	14.5
10	.31	v. rancid	18.3	v. rancid	21.0
14	.35	v. rancid	39.7	v. rancid	27.9
<i>21° C.</i>					
0	.12	good	.9	good	.9
4	.24	sl. rancid	7.0	sl. rancid	12.0
6	.36	rancid	13.2	rancid	17.3
10	.39	v. rancid	21.5	v. rancid	23.4
14	1.05	rancid, yeasty	42.0	v. rancid	32.6

TABLE 10  
Comparative lipolytic action of *O. lactis* in cream and in butter

Days held at	Cream			Butter	
	Per cent acidity	Flavor	Acid number	Flavor	Acid number
<i>5° C.</i>					
0	0.12	good	0.5	good	0.5
4	.21	old	1.5	good	.9
6	.23	old	5.7	old	2.3
10	.28	rancid	13.4	sl. old	3.5
14	.37	v. rancid	15.5	rancid	4.1
<i>13° C.</i>					
0	.12	good	.5	good	.5
4	.23	old	5.3	good	1.3
6	.29	old	8.3	sl. old	2.5
10	.42	rancid	15.3	sl. rancid	12.4
14	.57	v. rancid	19.4	rancid	14.8
<i>21° C.</i>					
0	.12	good	.5	good	.5
4	.32	rancid	8.2	old	11.0
6	.47	rancid	17.5	old	14.3
10	.68	v. rancid	31.3	v. rancid	33.4
14	1.05	v. rancid	43.0	v. rancid	38.5

#### EFFECT OF NEUTRALIZATION OF SOUR CREAM ON THE ACID NUMBER OF THE FAT

Raw cream was inoculated with a culture of a lipolytic organism, *Ach. lipolyticum*, and incubated until the acidity of the cream and the acid number of the fat had increased appreciably. To a series of quart jars, each

containing 1 pound of cream at 30° C., was added neutralizer in increasing amounts so that portions of cream at different acidities were obtained. Sodium carbonate and magnesium oxide were used. The cream was then pasteurized at 62° C. for 30 minutes and cooled. The lots of cream were churned and acid numbers of the fat were determined.

While the data (table 11) reveal a very definite reduction of the free acidity in the fat, the neutralization of this acidity was somewhat slower and less complete than the neutralization of the serum acidity. The free fatty acids were reduced in all samples of cream to which alkali was added but the rate of reduction was slow until the titratable acidity of the cream was reduced appreciably. Using sodium carbonate, comparatively little reduction was noted in the acidity of the fat until the titratable acidity of the cream had been reduced to 0.20 per cent; with magnesium oxide, to 0.15 per cent. After sufficient alkali had been added to reduce the cream to the neutral point using phenolphthalein as an indicator, some free acid still remained in the fat. Although there was no appreciable difference in the degree of reduction of the fatty acids by the two alkalies, magnesium oxide appeared to be slightly more effective than sodium carbonate. Results of experiments by Bird and Breazeale (1) show that a definite reduction in the fatty acids of cream occurred when it was neutralized.

TABLE II  
*Effect of neutralization of sour cream on acid number of the fat  
(Cream pasteurized at 62° C. for 30 minutes)*

Treatment of cream	Neutralized with			
	Sodium carbonate		Magnesium oxide	
	Per cent acidity in cream	Acid number of fat	Per cent acidity in cream	Acid number of fat
Raw . . . . .	0.72	2.1	0.72	2.1
Pasteurized, not neutralized	.71	1.9	.71	1.9
	.40	1.8	.44	1.8
	.29	1.8	.33	1.8
	.20	1.4	.27	1.7
Pasteurized and neutralized	.17	1.0	.15	1.2
	.13	.8	.11	1.2
	.10	.7	.10	1.2
	.00	.4	.00	

The work of Clark, *et al.* (2) included a study of the acid numbers of many samples of commercial butter of varying quality. They considered that a good correlation existed between the acid numbers of the fat of butter and the quality of the cream from which it was made and concluded that on the average, the poorer the quality the cream the higher the acid number of the fat of the resulting butter.

In general, the lots of high quality butter had low acid numbers on the

TABLE 12  
*Effect of neutralization of sour cream on the acid number of the fat of the resulting cream and butter  
 (All lots of cream from the mixed receipts of a local creamery)*

		(Turning number)									
		1	2	3	4	5	6	7	8	9	10
Unneutralized raw cream											
Flavor	sour	sour	sour	sour	sour	sour	sour	sour	sour; sl. bitter	sour; sl. runied	sour; off flavor
Per cent acidity	0.68	0.64	0.82	0.65	0.69	0.72	0.47	0.70	0.73	0.69	0.69
Acid number of fat	1.5	1.9	2.5	1.5	1.3	1.2	1.4	4.1	5.9	3.7	
Neutralized pasteurized cream											
Flavor	good	good	good	good	good	good	good	good	sl. bitter	old	old, off flavor
Per cent acidity	.25	.26	.25	.25	.27	.24	.26	.25	.24	.27	.23
Acid number of fat	1.0	1.4	1.4	1.5	1.4	1.0	1.1	1.2	1.3	1.4	1.2
Butter											
Flavor	coarse	coarse	coarse	acidy	old	coarse	clean, coarse	off flavor	old	old	coarse
Score	90	90.5	90	89.5	90	90	91	89.5	89.5	89.5	
Acid number of fat	.9	.9	1.0	1.0	1.0	.9	1.0	1.7	1.7	1.4	1.0

fat. It did not follow however that low quality cream always produced butter with the higher acid numbers on the fat. Poor quality butter made from neutralized cream, often showed a relatively low acid number, depending on the degree of fat hydrolysis, the type and amount of neutralizer used in the cream. Judging from these data and from the results of others (1), one is not justified in assuming that butter with a low acid number on the fat was made from good quality cream.

A study was made on cream delivered to a local creamery, as related to the effect of the neutralization process on the acid number of the butterfat. In each trial the cream was received at the creamery, weighed, sampled and dumped into the pasteurizing vat without regard to quality. When the vat was full, the cream was warmed to about 30° C., a sample of the mixed cream was removed from the vat and immediately cooled. The acidity of the cream in the vat was then standardized to approximately 0.25 per cent with a lime neutralizer. After the cream had been pasteurized and cooled a second sample was removed from the vat. A sample of butter was also taken from the completed churning. The samples of cream were churned and the acid numbers of the fat of the cream as well as of the butter were determined.

The titratable acidities of the unneutralized cream (table 12) varied from 0.47 to 0.82 per cent and the acid numbers of the fat from 1.2 to 5.9. After standardization of the cream with alkali to acidities ranging from 0.23 to 0.27 per cent, the acid numbers of the fat ranged from 1.0 to 1.5. The acid numbers of the fat of the resulting butter ranged from 0.9 to 1.7. In every instance the acid number of the fat was reduced when the acidity of the cream was standardized. While there was considerable variation in the acid numbers of the fat of the unneutralized cream, the acid numbers after neutralization were comparatively uniform. This indicates that the percentage reduction of the fat acidity due to neutralization was considerably greater in the samples of raw cream with high acid numbers than in the samples with low acid numbers. The cream with the high acid numbers before neutralization made slightly lower quality butter than the cream with low acid numbers. Samples 8, 9 and 10 had higher acid numbers than the other samples and the resulting butter was of slightly lower quality.

#### CONCLUSIONS

1. Of the two biological agencies causing fat hydrolysis in raw cream, organisms were found to be of greater significance than lipase.
2. In raw cream containing no formaldehyde, in which both lipase and micro-organisms were active, the lipolysis was greater at 5° than at 13° or 21° C.; in cream containing formaldehyde, in which lipase only was active, the degree of hydrolysis increased as the holding temperature of the cream increased within the range studied.
3. The growth of butter culture organisms or *L. bulgaricus* in sterilized

cream, resulting in titratable acidities ranging up to 0.89 per cent with the former and up to 2.02 per cent with the latter, failed to cause changes in the acid numbers of the fat.

4. With the exception of *Ps. fragi*, all organisms studied which showed lipolysis on agar plates containing fat caused increases in the acid numbers of the fat when inoculated into sterilized cream, although rancidity did not result in every instance.

5. All organisms studied were more actively lipolytic in cream than in butter, especially at 5° C.

6. When the titratable acidity of sour cream was reduced by the addition of an alkali, the acid number of the fat was also reduced, but not proportionately.

7. Because of the decrease in the acid number of fat resulting from the neutralization process, it cannot be assumed that butter with a low acid number on the fat was made from good quality cream.

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# LIVE WEIGHT AND MILK-ENERGY YIELD IN HOLSTEIN COWS

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This paper extends an analysis of the relation between live weight and milk-energy yield in cows, based on data available in the literature (*cf.* 1; 2). The records considered here are from the Cornell Station (3, 4) and from the Bureau of Dairy Industry (5). The former pertain to grade Holstein cows of mature age; the latter, to pure-bred half-sister Holstein cows as first-calf heifers and at mature age.

## CORNELL DATA

These represent a feeding trial designed to test the effect of concentrate rations differing in per cent of total protein for 3 successive seasons. They include the initial live weight of each cow and her milk and fat yield for the first 40 weeks of lactation in the first and second seasons and the first 37 weeks in the third season.

*Initial live weight (IW) and milk energy (FCM).* The correlations between initial live weight and milk energy per unit live weight work out as follows:

First season, 32 cows,  $IW = 1084$  to  $1333$  pounds,  $r = -.26 \pm .11$   
Second season, 36 cows,  $IW = 1120$  to  $1503$  pounds,  $r = -.06 \pm .11$   
Third season, 35 cows,  $IW = 1062$  to  $1534$  pounds,  $r = -.02 \pm .11$

These results suggest a progressive change in the relation of initial live weight to milk-energy yield. Inasmuch as the cows were purchased in the open market shortly before start in the trial it may be inferred that the management in the station herd develops a condition of substantial independence between initial live weight and milk-energy yield per unit initial live weight. That is, milk energy tends to become a multiple of initial live weight under favorable conditions of management.

Twenty-two of the cows were in the trial all 3 seasons. If a progressive change occurs as above suggested it should be indicated more trustworthily by these 22 cows considered by themselves. The correlations for the 22 cows are:

First season,  $IW = 1084$  to  $1308$  pounds,  $r = -.15 \pm .14$   
Second season,  $IW = 1120$  to  $1503$  pounds,  $r = +.16 \pm .14$   
Third season,  $IW = 1163$  to  $1534$  pounds,  $r = +.11 \pm .14$

Apparently such change as may occur is brought about during the first lactation and rest period in the experiment. As between the cows in this group of 22 during the second and third seasons the larger cows at the start

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of lactation produce more milk energy per unit size than the smaller cows. If milk energy is expressed as a power function of initial live weight the exponent is greater than unity.

The relation between initial live weight and milk-energy yield for these 22 cows is shown graphically in figure 1.

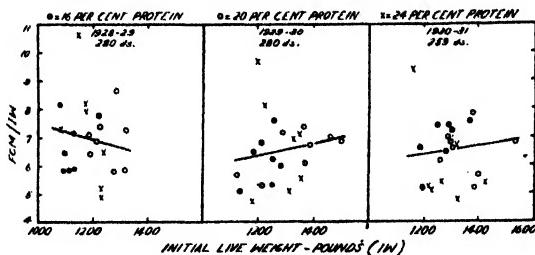


FIG. 1. Relation of milk-energy yield in pounds of 4 per cent milk per pound initial live weight ( $FCM/IW$ ) to initial live weight in pounds (IW).

Equations of the straight lines:

$$1928-29, FCM/IW = 10.12 - .00265 IW$$

$$1929-30, FCM/IW = 3.99 + .00197 IW$$

$$1930-31, FCM/IW = 4.63 + .00141 IW$$

*Effect of per cent protein in the concentrates.* The general conclusion (3, 4) from the experiment was that there was little difference between the 3 rations as to effect on milk yield. However, on the basis of  $FCM/IW$  for the 22 cows in the trials all 3 seasons there appears to be an appreciable and consistent difference in favor of the high-protein concentrates. Table 1 shows the results on this basis. In both the first and second seasons the group average  $FCM/IW$  increases with the per cent of protein in the concentrates. In the third season the 16 per cent group, which had received the concentrate with 16 per cent protein continuously in the first and second seasons, was started on 16 per cent concentrates for 5 weeks and then alter-

TABLE 1

*Initial live weight and milk-energy yield per unit initial live weight by groups according to the per cent of protein in the concentrates fed*

(From publications (3, 4) of the Cornell Station)

Group	Number of cows	1928-29, 280 days		1929-30, 280 days		1930-31, 259 days	
		Mean IW	Mean $FCM/IW$	Mean IW	Mean $FCM/IW$	Mean IW	Mean $FCM/IW$
16 per cent .....	7	1125	6.74	1224	6.23	1266	6.87
20 per cent .....	8	1257	6.93	1326	6.54	1358	6.60
24 per cent .....	7	1175	7.24	1276	6.76	1269	6.00

IW = initial live weight in pounds, determined about 3 days after calving.

FCM = milk-energy yield in pounds of 4 per cent milk =  $.4 \times$  pounds milk +  $15 \times$  pounds fat. Milk energy in calories =  $340 \times FCM$ .

nated with 24 per cent and then 20 per cent by 5-week periods. A similar procedure was followed for the other two groups. Hence the results are not comparable in the same way as for the first and second seasons.

If milk-energy yield per unit initial live weight is a fair basis of comparison it appears there may be some increase in yield associated with high-protein feeding for the first and second seasons. Results for the third season suggest residual effects. It is the purpose here merely to mention the idea of using FCM/IW as a measure of yield in the first and second seasons.

#### BUREAU OF DAIRY INDUSTRY DATA

These records pertain to a breeding project in which a consideration of first importance is a quantitative determination of the milking capacity of the cows for genetic analysis. The Bureau plan is to test each cow with her first calf and again after 5 years of age; feed liberally; milk 3 times daily; breed not earlier than the fifth month of lactation; and use 365 days as the test period. The records are therefore on quite a different plane than those of the Cornell experiment utilized above. The record of live weight is an average of 24 semi-monthly weighings during the test period (in contrast to initial live weight in the Cornell data).

The data considered here are the records of 32 daughters (excluding no.

TABLE 2

*Coefficients of correlation (r) in the records of 32 daughters of a Holstein bull  
(From a publication (5, table 11) of the Bureau of Dairy Industry)*

Variables correlated	n	r
A. Records of the same cow or different cows with respect to:		
1. Live weight and milk energy per unit live weight	61	$-.274 \pm .080$
2. Live weight and milk energy per unit live weight*	34	$+.136 \pm .118$
3. Live weight and milk energy per unit live weight†	27	$-.104 \pm .129$
4. Live weight and age-corrected milk	61	$-.117 \pm .085$
5. Age and milk energy per unit live weight	61	$-.013 \pm .086$
6. Age and age-corrected milk	61	$-.073 \pm .086$
7. Milk energy and age-corrected milk	61	$+.679 \pm .046$
8. Milk energy and milk energy per unit live weight	61	$+.745 \pm .038$
9. Milk energy per unit live weight and age-corrected milk	61	$+.813 \pm .029$
B. First and last records of the same cow with respect to:		
1. Fat percentage	22	$+.624 \pm .088$
2. Milk energy per unit live weight, FCM/W	22	$+.697 \pm .074$
3. Milk energy, FCM	22	$+.725 \pm .068$
4. Milk	22	$+.772 \pm .058$
5. Age-corrected milk	22	$+.826 \pm .046$
C. One record and another of the same cow with respect to:		
1. Fat percentage	72	$+.584 \pm .053$
2. Milk energy per unit live weight, FCM/W	72	$+.692 \pm .042$
3. Milk energy, FCM	72	$+.294 \pm .073$
4. Milk	72	$+.382 \pm .068$
5. Age-corrected milk	72	$+.760 \pm .034$

\* Records below 1323 pounds live weight, the mean of the 61 records.

† Records above 1323 pounds live weight, the mean of the 61 records.

803) of the Holstein bull, Denton Colantha Sir Rag Apple, at Beltsville as published (5) in table 11.

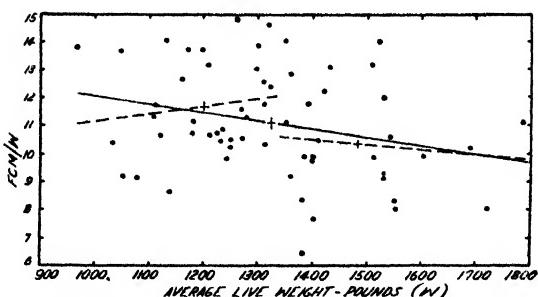


FIG. 2. Relation of milk-energy yield in pounds of 4 per cent milk per pound average live weight (FCM/W) to average live weight in pounds (W). The crosses indicate the means.

Equations of the straight lines:

$$\text{All records} \dots, \text{FCM}/\text{W} = 15.11 - .00304 \text{ W}$$

$$\text{Records above average W, FCM}/\text{W} = 12.96 - .00177 \text{ W}$$

$$\text{Records below average W, FCM}/\text{W} = 8.75 + .00244 \text{ W}$$

*Average live weight (W) and milk energy (FCM).* Figure 2 presents the correlation surface for W and FCM/W for 61 records of the 32 daughters. The coefficient of correlation is -.274, table 2. Inspection of figure 2 shows an upward trend of FCM/W against W in the left half of the surface. In fact, 34 records below average weight (1323 pounds) give a correlation of +.136; while 27 records above average weight give  $r = -.104$ , table 2. Considered over the entire range of live weight FCM/W appears to bear a curvilinear relation to live weight.

The authors (5, p. 7) feel that some of their highest-producing cows could have made still greater records with 4 daily milkings, artificial aids to cooling of the hard-working, heat-producing organism during the hot days of summer, etc. Since the highest-producing cows tend to be found among the largest cows we may infer that the full capacity of the largest cows was not realized, and had it been we might find substantially a horizontal trend of FCM/W against W for the whole range of W. Without artificial cooling there are times when an 1800-pound cow, as compared with a 900-pound cow, is decidedly handicapped by the work of getting rid of surplus heat.

As the record stands the daughters of this bull seem to have inherited a very strong proclivity to lactation, manifest not alone in the absolute milk energy produced but also in the relation of milk-energy yield to live weight. Figure 3 affords a direct graphic demonstration of how well the individual observations conform to a simple-multiple relation between live weight and milk-energy yield.

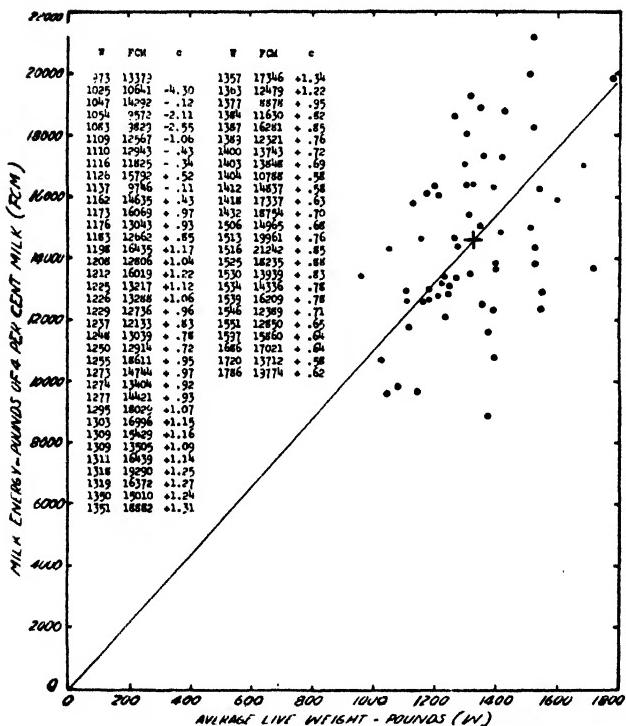


FIG. 3. Relation of milk-energy yield in pounds of 4 per cent milk (FCM) to average live weight in pounds (W).

The straight line is drawn from the origin through the means (cross) and has the equation,  $FCM = 11.02 W$  (mean  $FCM = 14581$ , mean  $W = 1323$ , and  $14581/1323 = 11.02$ ).

The numerical data on the chart give the observations arranged in order of live weight and fitted with the equation,  $FCM = bW^c$ , by live-weight stages. For the lowest two live weights  $c = -4.30$ ; for the lowest 3,  $c = -.12$ ; and so on to all 61 observations where  $c = +.62$ . For live weights from 973 to 1116 pounds, inclusive, milk-energy yield is inversely proportional to the .34 power of live weight. This may be interpreted to mean that there is a tendency for the cows with high rate of yield to have low rate of gain in live weight, thus tending to associate high yield with low-average live weight. The use of initial live weight, instead of average live weight, might eliminate this difficulty. (The published data do not include initial live weight.) For live weights from 973 to 1357 pounds, inclusive, milk-energy yield is proportional to the 1.34 power of live weight. Beyond this point there are rapid declines in the value of  $c$ , due to several exceptionally low yields per unit live weight. This may be interpreted to indicate a relative environmental handicap for the large cows. These interpretations consider that age of the cow is of itself a practically negligible factor in her milk-energy yield.

*Age correction vs.  $FCM/W$ .* The age corrections applied to milk yield (5, p. 25) "are a composite of the available adjustment factors." (The number of sets of factors in the composite is not stated but it must be large

to include all of those available.) As shown in table 2 the correlation between age and age-corrected milk is  $-.07$ . That is, the correction effectively reduces the age trend to a horizontal line.

The correlation between age and FCM/W is  $-.01$ . That is, FCM/W, a direct factual measure of yield, is even more independent of age than is age-corrected milk.

The question may be raised, is age correction an indirect way of allowing for live weight, or is FCM/W an indirect way of allowing for age? A definite answer is afforded by the data of Kleinberg previously cited (2), representing some 14,000 annual records. When live weight was held within a narrow range age had no influence on milk yield. On the other hand when age was held within a narrow range, live weight had practically the same effect on milk yield as when age was not held constant. It may be concluded that age correction is an indirect way of allowing for live weight.

While age-corrected milk and FCM/W are closely related,  $r = .813$ , the two things are by no means identical or the same in meaning. The close association arises in the condition that both are largely dependent on the absolute milk-energy yield, as evidenced by the correlations given in table 2, A7 and A8.

*Correlation between records of the same cow.* Dealing with the first and last records of the same cow, with respect to the same item, table 2, section B, the items and correlations, in increasing order of  $r$  follow: fat percentage,  $.62$ ; FCM/W,  $.70$ ; FCM,  $.73$ ; milk,  $.77$ ; age-corrected milk,  $.83$ . What is the meaning of high and low values of  $r$  in such a comparison?

Let us consider fat percentage to illustrate one point of view in answer to the question. If the 22 cows are inherently exactly alike fat percentage will fluctuate at random, from non-inherent influences, and the correlation will tend to be zero; if the cows are inherently or consistently unlike the correlation will tend to be one. Conversely, a low value of  $r$  indicates likeness (presumably genetic homogeneity) in the cows, while a high value of  $r$  indicates unlikeness in the cows, with respect to the character under consideration. As an illustration, the 22 grade Holstein cows, at Cornell, table 1, purchased on the market without regard to relationship, show a correlation for the first and last records with respect to fat percentage of  $r = .93$ , in contrast to  $r = .62$  for the comparable correlation in the 22 pure-bred Holstein cows at Beltsville, table 2, all daughters of the same sire. This example, at least, seems consistent with the above interpretation of the meaning of high and low values of  $r$  in the correlation between the first and last records of the same cow with respect to fat percentage.

Age correction changes the correlation for milk from  $.77$  to  $.83$ , that is, makes the cows more unlike. Dividing by  $W$  changes the correlation for FCM from  $.73$  to  $.70$ , that is, makes the cows more alike. Unfortunately,

there seems no way to know which direction of change better fits the true condition of likeness.

#### SUMMARY

Sixty-six records of 22 grade Holstein cows in a feeding experiment at Cornell for 3 successive seasons, show that milk-energy yield per unit initial live weight decreased with live weight during the first season, and increased with live weight during the second and third seasons. It is concluded that milk-energy yield tends to be a multiple of initial live weight (3 days after calving) under continued favorable, practical conditions.

Sixty-one records of 32 daughters of the same Holstein bull in a breeding experiment at Beltsville show that milk-energy yield per unit average live weight decreased with live weight; 27 of the 61 records above average live weight show a similar tendency; 34 of the 61 records below average live weight show an opposite tendency. The 61 records show milk-energy yield per unit live weight is independent of age ( $r = -.01 \pm .09$ ); age-corrected milk is independent of age ( $r = -.07 \pm .09$ ). It is concluded that age-correction is an indirect way of allowing for live weight.

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# THE VIABILITY OF SEEDS AS AFFECTED BY THE SILOING PROCESS

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Seeds of some of the farm crops and of the more common weeds were placed in the silo along with corn or alfalfa at the time of filling. When the silage was fed down to the seeds they were removed, dried at room temperature, and tested for germination by the Division of Seed Investigations, Bureau of Plant Industry. Twenty-nine lots (or kinds) of seed were buried in silage for the study. Duplicate samples of 22 lots were stored in the office and tested for germination to compare their viability with that of the samples in the silo. Detailed results of this work are shown in the following tables.

TABLE 1

*Viability of seeds buried in alfalfa silage and in grass-and-alfalfa silage, and of similar seeds stored in the office*

Name of seed	Place stored	Duration of test	Germination	Condition of remaining seeds at the close of the test
(Seeds in alfalfa silage with 34 per cent moisture, 14 feet below the surface, from June 1, 1934, to January 18, 1935)				
Shepherd's purse <sup>1</sup> .....	Silo	Days	Per cent	Many sound Many sound
	Office	43	27	
Chickweed <sup>2</sup> .....	Silo	24	0	
	Office	24	97	
(Seeds in grass-and-alfalfa silage with 45 to 50 per cent moisture from May 28, 1936, to July 25, 1936)				
Chickweed .....	Silo	Days	Per cent	
	Office	63	88	
Buttercup <sup>3</sup> .....	Silo	49	0	
	Office	49	87	
Dandelion <sup>4</sup> .....	Silo	21	0	
	Office	21	52	
Shepherd's purse <sup>1</sup> .....	Silo	28	0	
	Office	12	96	
Timothy <sup>5</sup> .....	Silo	8	0	
	Office	8	77	

<sup>1</sup> *Bursa bursa-pastoris*.

<sup>4</sup> *Taraxacum officinale*.

<sup>2</sup> *Alsine media*.

<sup>5</sup> *Phleum pratense*.

<sup>3</sup> *Ranunculus* sp.

<sup>6</sup> *Avena sativa*.

When the 29 lots of seed were removed from the silo and tested, all seeds failed to germinate except some of those in the samples of *Lespedeza sericea*,

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TABLE 2

*Viability of seeds buried in corn silage, and of similar seeds stored in the office  
(Seeds in corn silage with normal moisture content, 30 feet below surface, from October  
5, 1936, to April 4, 1937)*

Name of seed	Place stored	Duration of test	Germination	Condition of remaining seeds at the close of the test
		Days	Per cent	
Bigseed ladys-thumb or Pennsylvania smartweed <sup>1</sup> .....	Silo	148	0	4 per cent sound 41 per cent sound
	Office	148	37	
Goose grass <sup>2</sup> .....	Silo	21	0	
	Office	21	97	
Amaranth <sup>3</sup> .....	Silo	148	0	21 per cent sound
	Office	127	68	
Crabgrass <sup>4</sup> .....	Silo	56	0	Decayed
	Office	56	85	
Common ragweed <sup>5</sup> .....	Silo	117	0	None sound
	Office	148	74	
Pigeon grass or foxtail <sup>6</sup> .....	Silo	149	0	
	Office	148	93	

<sup>1</sup> *Polygonum pensylvanicum*.

<sup>2</sup> *Eleusine indica*.

<sup>3</sup> *Amaranthus sp.*

<sup>4</sup> *Digitaria sp.*

<sup>5</sup> *Ambrosia artemisiifolia*.

<sup>6</sup> *Setaria sp.*

bindweed, and American dragonhead mint. There was a high percentage of hard seeds in the samples of *Lespedeza sericea* and bindweed and a very small percentage in the samples of sweetclover seed that survived the ensiling process and the germination test.

Some seeds germinated in all the 22 lots that were kept in the office, except in those of perennial sowthistle and Canada thistle, and even these two lots were known to be viable shortly before they were placed in the silo. Although no definite statement can be made regarding the viability of the 7 lots for which no duplicates were kept in the office, it should be noted in table 1 that one sample of shepherd's purse seed kept in the office germinated to the extent of 27 per cent, which indicates that the other sample of shepherd's purse might likewise have contained some viable seed. Also, in the 1938 trial no chickweed was kept in the office (table 3), but in previous trials two samples kept in the office had a high percentage of germination (table 1). Our general knowledge of three others—*Lespedeza sericea*, Johnson grass, and sweetclover—would lead us to believe that these would show some viability after being kept in the office. Some of the two remaining lots of seeds—Indian mustard and American dragonhead mint—were successfully used to raise plants in a greenhouse shortly before seeds were placed in the silo. It appears reasonable to assume, therefore, that they would have shown some viability if they had been tested after storage in an office.

Silages of corn, and grass and alfalfa all destroyed the viability of the seeds and while there is no way of estimating the relative effectiveness of the different silages in this respect, there is a slight indication that the low-

TABLE 3  
Seeds in alfalfa silage with different moisture contents and preserved with and without molasses compared with seeds kept in the office.  
Seeds placed in the silo June 15<sup>38</sup>

Name of seed	Dura- tion of test	32 per cent moisture no molasses 6 feet below surface removed December 1938		41 per cent moisture 4.18 per cent molasses 14 feet below surface removed January 1939		48.5 per cent moisture no molasses 15½ feet below surface removed January 1939	
		Germination	Condition of remaining seeds at close of test	Germination	Condition of remaining seeds at close of test	Germination	Condition of remaining seeds at close of test
Quackgrass <sup>4</sup>	21	0	Decayed	0	Decayed	0	Decayed
Corn cockle <sup>5</sup>	14	0	"	0	"	0	"
India mustard <sup>6</sup>	7	0	"	0	"	0	"
Perennial sowthistle <sup>7</sup>	14	0	"	0	"	0	"
Oxeye daisy <sup>8</sup>	14	0	"	0	"	0	"
Chickweed <sup>9</sup>	21	0	"	0	"	0	"
Johnson grass <sup>10</sup>	28	0	"	0	"	0	"
Lespedeza sericea <sup>11</sup>	21	1	56% hard seed; rest decayed	5	47% hard seed; rest decayed	2	60% hard seed; rest decayed
Bindweed <sup>12</sup>	28	11	40% hard seed; rest decayed	9	37% hard seed; rest decayed	5	44% hard seed; rest decayed
Sweet clover <sup>13</sup>	7	0	No hard seed; decayed	0	1% hard seed; rest decayed	0	1% hard; rest decayed
American dragonhead mint <sup>14</sup>	28	3		36		0	Decayed
Canada thistle <sup>15</sup>	14	0	Decayed	0	Decayed	0	"
Horse nettle <sup>16</sup>	14	0	"	0	"	0	"
Leaty spurge <sup>17</sup>	14	0		0		0	"
Perennial Pepper grass or white-top <sup>18</sup>	14	0	"	0	"	0	"

<sup>1</sup> Most of these seeds were furnished by Mr. L. W. Kephart,  
Bureau of Plant Industry.

<sup>2</sup> *Agropyron repens*.

<sup>3</sup> *Agrostemma githago*.

<sup>4</sup> *Brassica juncea*.

<sup>5</sup> *Sonchus arvensis*.

<sup>6</sup> *Chrysanthemum lecanthemum pinnatifidum*.

<sup>7</sup> *Alsin media*.

<sup>8</sup> *Sorghum halepense*.

TABLE 3.—(Continued)

Name of seed	Duration of test	69 per cent moisture no molasses 17 feet below surface removed February 1939		65 per cent moisture 3.44 per cent molasses 21 feet below surface removed March 1939		Kept in office	
		Germi- nation	Condition of remaining seeds at close of test	Germi- nation	Condition of remaining seeds at close of test	Duration of test	Germi- nation
Quackgrass <sup>9</sup>	21	0	Decayed	0	Decayed	Days	Per cent
Corn cockle <sup>10</sup>	14	0	“	0	“	30	22
India mustard <sup>11</sup>	7	0	“	0	“	21	87
Perennial sowthistle <sup>12</sup>	14	0	“	0	“	No test made	Decayed
Oxeye daisy <sup>13</sup>	14	0	“	0	“	21	0
Chickweed <sup>14</sup>	21	0	“	0	“	30	51 & 30
Johnson grass <sup>15</sup>	28	0	“	0	“	No test made	Decayed
Lespedeza sericea <sup>16</sup>	21	1	62% hard seed; rest decayed	0	67% hard seed; rest decayed	Days	Per cent
Bindweed <sup>17</sup>	28	6	49% hard seed; rest decayed	5	53% hard seed; rest decayed	30	53% hard seed;
Sweet clover <sup>18</sup>	7	0	1% hard seed; rest decayed	0	1% hard seed; rest decayed	21	22
American dragonhead mint <sup>19</sup>	28	0	Decayed	0	Decayed	No test made	rest decayed
Canada thistle <sup>20</sup>	14	0	“	0	“	21	“
Horse nettle <sup>21</sup>	14	0	“	0	“	30	55
Leafy spurge <sup>22</sup>	14	0	“	0	“	30	59
Perennial pepper grass or white-top <sup>23</sup>	14	0	“	0	“	30	“

- <sup>9</sup> *Lepidium sativum*.  
<sup>10</sup> *Convolvulus arvensis*.  
<sup>11</sup> *Melilotus officinalis*.  
<sup>12</sup> *Dracontium parviflorum*.

<sup>13</sup> *Cirsium arvense*.<sup>14</sup> *Solanum carolinense*.<sup>15</sup> *Euphorbia Esula*.<sup>16</sup> *Lepidium draba*.

moisture silages may not be quite so effective in destroying the viability as high-moisture silages.

One should not overlook the fact that at the conclusion of the germination test some of the seeds were rated as "sound" or "hard." Such seeds may or may not have germinated subsequently, if they had been subjected to conditions different from those under which these tests were made.

It is well known that when a weedy crop is made into hay, many weed seeds are preserved in a viable condition, also that the application on the land of stable or barnyard manure, at least that which has not been composted or allowed to rot, usually increases the prevalence and growth of weeds. It appears, therefore, that the conversion of weedy crops into silage instead of into hay will help materially in the control of weeds.



## American Dairy Science Association Announcements

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Thirty-Fifth Annual Meeting, Purdue University, West Lafayette, Indiana, June 24-28, 1940. Information concerning rooms and housing will be given in the April issue of this Journal.

### CALL FOR TITLES AND ABSTRACTS

All abstracts to be presented at our annual meeting in June must be in our hands by April 15. Please send titles and abstracts to Dr. B. E. Horrall, Department of Dairy Husbandry, Purdue University, West Lafayette, Indiana.



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## ACID VALUES AND ACID RATIOS AS RELATED TO THE KEEPING QUALITY OF SALTED BUTTER<sup>1</sup>

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Since acidity affects the activity of micro-organisms and enzymes as well as the speed of purely chemical reactions, it would seem that the amount of acid present or developing in butter when held for one week at 21° C. might give an indication of the keeping quality of the butter. It was realized that the influences of acidity may exert themselves in different directions, but in order to study the relationships which might most commonly exist between acidity and the keeping quality of salted butter, especially sweet cream butter, when held for one month at 0-5° C., a number of samples of commercial butter obtained from various Washington creameries were examined for the acid values of both the butter and the butterfat and for the ratio of butterfat acidity to butter acidity. The latter will be referred to as the acid ratio.

### REVIEW OF LITERATURE

That various undesirable bacteria are retarded in their growth by the presence of considerable amounts of acid in butter has been shown by many investigations. Thus, Collins and Hammer (5) when growing lipolytic bacteria on Nile-blue sulphate agar of pH values of 5.3, 6.7, and 7.8 noted that with many of the organisms the most alkaline reaction seemed to favor the hydrolysis of simple triglycerides and natural fats. Grimes (13) concluded that the acidity of ripened butter inhibited the growth of proteolytic bacteria. Sadler and Vollum (26) showed that much more deterioration occurred in butter made from overneutralized cream than in butter from cream of 0.25 per cent acidity or over when inoculated with the bacteria obtained from deteriorated butter. Guthrie, Scheib, and Stark (15) found no significant numbers of proteolytic and lipolytic bacteria in sour cream butter. On the other hand yeast and mold growth is known to be favored by acidity.

Most enzymes seem to be less active in the presence of acid. Thus, Davies

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<sup>1</sup> Published as Scientific Paper No. 412, College of Agriculture and Experiment Station, State College of Washington.

(6) states that the acidity of ripened cream prevents lipase action even in raw cream. Dorner and Widmer (7) found that the enzyme lipase was inhibited by an acid reaction and according to Babcock, Russell, and Vivian (2) galactase also is retarded by acidity. Davies (6) and Rogers (23) reported the enzyme galactase to be most active in the pH range of 6.4-7.2 at temperatures of 37-42° C. The most active proteolytic enzymes were found by Spitzer, Parfitt, and Epple (28) to be produced by *Bacillus ichthyosmius* and *Achromobacter putrefaciens* and these enzymes seemed to be most active at a pH of 7.0 and greatly reduced in activity at pH 4.0 and 3.0. According to Davies (6) catalase is also slightly inhibited by acid conditions while peroxidase is active over a wide range of pH. However, he states that the inactivation of catalase by heat seems to be retarded by acidity and Zilva (29) found this to be true also in the case of peroxidase. Guthrie, Scheib, and Stark (15) concluded that the action of all enzymes was inhibited by a pH of 4.4-4.64 in unsalted butter.

In the light of the above-mentioned investigations, it remains very doubtful, however, whether the normally low acidity of sweet cream butter exerts any significant retarding effect on the action of undesirable bacteria or enzymes. On the other hand, an acidity which is comparatively high for sweet cream butter may be a general indication of high bacterial contamination.

Rogers and Gray (24) decided that the deleterious effect of lactic acid in producing fishy butter was not due to any organism, enzyme or other substance which would be destroyed by pasteurization at 77-82° C. by the flash method or at 70° C. for 10 minutes, but that acidity itself was responsible. Dyer (8) showed that the development of undesirable flavors in butter held in cold storage at -17.8° C. was due to the oxidation of non-fatty substances in the butter and that the extent of this chemical change was directly proportional to the quantity of acid present in the cream from which the butter was made. Greenbank and Holm (12) concluded that increases in the acidity of fat increase its susceptibility to oxidation. That the production of trimethylamine, the cause of fishy flavors in butter, is accelerated by the presence of acid was shown by Sommer and Smit (27).

According to Loftus-Hills, Scharp, and Bellair (18) high acidity and a low pH correlated rather closely with low keeping quality in sweet cream butter. Similar results were obtained by Arup and Gilmour (1), Gilmour (10), and Gilmour and Arup (11). The latter investigators concluded that a consideration of pH values was more valuable in selecting butter for cold storage than a knowledge of the acid values, and that butter with a pH of over 6.7 kept better than butter of a lower pH.

Bouska (4) stated that butter tends to be low in keeping quality at a pH of less than 6.0, keeps better at a pH of 6.0-6.8, develops surface flavors readily at pH 7.0, and tallowy flavors at a higher pH.

The results of Patrick, Leighton, and Bisbee (21), Patrick, Leighton,

and Heileman (22), Rogers, Thompson, and Keithley (25), Mortensen (19), and Grimes (13) (14) all indicated that the butter of lowest acidity, whether made from raw or pasteurized cream, had the best keeping quality at various storage temperatures up to 10° C. Larson, Lund, and Miller (17) noticed that the percentage of acid found in the fat increases with the age and rancidity of the butter. Frielinghaus (9) examined many samples of butter for their acid ratio which is the ratio of the acid value of the butterfat to the acid value of the butter. He found that the acid ratio of butter made from cream inoculated with yeasts gradually increased during storage of the butter for 20 days at 4° C. At the same time the flavor became increasingly stronger. He obtained similar results when inoculating the cream with cladosporium and concluded that in the development of rancid flavors the acid ratio will increase, while in the development of high acid flavors it will decrease. When tallowy flavors developed, both the acid values of the butter and of the butterfat were raised, leaving the acid ratio little changed.

#### METHODS USED

Acid values were determined on 28 samples of sweet cream butter, 51 samples of neutralized cream butter without butter culture, and 8 samples of neutralized cream butter with butter culture, and also on the butterfat separated from these samples. The determinations were made when the samples were fresh, after a week at 21° C., and after a month at 0-5° C.

##### *Acid values of butter*

The butter was prepared for titrating by macerating 70-80 grams in a clean, dry cup by means of a spatula until it appeared glossy and salty.

A 10-gram sample was weighed into a tared porcelain casserole or Erlenmeyer flask on a torsion butter moisture balance. Then 50 ml. of ethyl alcohol, neutral to phenolphthalein, and a few drops of phenolphthalein were added and the mixture brought to a boil on an electric hot plate. The boiling hot mixture was titrated with N/50 NaOH while being stirred constantly. The number of milliliters of N, 50 NaOH used to produce a pink color stable for at least one minute was taken as the acid value. This is essentially the method recommended by Bird and Breazeale (3).

##### *Acid values of butterfat*

The butterfat was obtained by filling a 50 ml. centrifuge tube with butter, melting it in a water bath at 40-45° C. and centrifuging the tube of melted butter. A sample of 10 grams of the unfiltered oil was titrated immediately by the procedure described above for butter in order to determine its acid value.

##### *Acid ratios*

The acid ratios were determined by dividing the acid values of the butterfat by those of the butter and multiplying by 100.

*pH values of butter serum*

The pH of the serum was determined at 25° C. by means of a Leeds and Northrup quinhydrone pH indicator (catalog No. 7654), using a rectangular plate gold electrode together with a saturated calomel half-cell and a saturated KCl agar bridge. The butter serum was obtained by centrifuging the butter melted at 40–45° C. and removing the butterfat.

Table 1 gives the average, maximum and minimum acid values converted into percentages of acid, calculated as lactic acid. The maximum acidity

TABLE I

*Maximum, minimum and average acidities found in butter and butterfat and calculated from the acid values as percentages of lactic acid*

	Per cent acidity calculated as lactic acid in						H ion concentration (as pH)	
	Butter			Butterfat				
	When fresh	After 1 week at 21° C.	After 1 month at 0–5° C.	When fresh	After 1 week at 21° C.	After 1 month at 0–5° C.		
I. Sweet cream butter (28 samples)								
Maximum .....	0.170	0.203	0.169	0.115	0.152	0.131	5.8	
Maximum* .....	0.130	0.145	0.131	0.113	0.115	0.112	6.0	
Minimum .....	0.070	0.072	0.079	0.038	0.045	0.051	6.8	
Average .....	0.095	0.109	0.103	0.067	0.076	0.078	6.42	
II. Neutralized cream butter without butter culture (51 samples)								
Maximum .....	0.178	0.207	0.176	0.129	0.203	0.152	6.0	
Minimum .....	0.072	0.079	0.083	0.038	0.050	0.063	7.6	
Average .....	0.122	0.140	0.132	0.084	0.102	0.099	6.50	
III. Neutralized cream butter with butter culture (8 samples)								
Maximum .....	0.160	0.198	0.167	0.162	0.182	0.157	5.6	
Minimum .....	0.110	0.119	0.144	0.070	0.065	0.098	7.0	
Average .....	0.138	0.158	0.155	0.096	0.116	0.125	6.33	

\* Considering only the 26 samples with pH values of 6.0 or over.

found in fresh sweet cream butter was 0.130 per cent, and in the butterfat of such butter 0.113 per cent. The maximum acidity found in these samples after storage, either for 1 week at 21° C. or for 1 month at 0–5° C. was 0.145 per cent in the butter and 0.115 per cent in the butterfat. The minimum acidity encountered was 0.070 per cent in this type of butter and 0.038 per cent in the butterfat.

Sweet cream butter had a lower minimum acidity and also a lower average acidity than the neutralized cream butter, even though the maximum pH found for sweet cream butter was 6.8 and for neutralized cream butter 7.6. Such observations harmonize with the results of Hunziker and Cordes (16) who found that sweet cream produced butter of a decidedly

lower pH than the same cream when soured, neutralized and then churned at the same acidity as the sweet cream.

Nissen (20) reported the titratable acidity of butter in an aqueous mixture to range from 0.02 to 0.04 per cent, if made from cream of about 0.15 per cent acidity, and from 0.03 to 0.05 per cent if made from cream testing 0.25 per cent in acidity. The values in an alcoholic mixture are always considerably higher than those in an aqueous mixture, probably because of the release of fatty acids from the fat by the alcohol. The pH at the end point of the butter titration in alcohol as conducted in this study usually was 7.8-7.95 compared with 7.35-7.73 as reported by Nissen in his titrations in water.

### *Acid values and keeping quality*

An examination of the data obtained in this study revealed no close relationship between the individual acid values of the fresh butter, the fresh butterfat, or either of these after storage for 1 month at 0-5° C. or for 1

TABLE 2

*The increase in the acid value of butterfat during 1 week at 21° C. as related to the keeping quality of salted butter when held for 1 month at 0-5° C.*

Number of samples	Increase in acid value of butterfat during 1 week at 21° C.	Average loss in score during 1 month at 0-5° C.	Average score after 1 month at 0-5° C.
I. Sweet cream butter			
6 .....	Below-0	0.58	36.42
8 .....	0 -0.45	1.06	36.00
6 .....	0.5-0.95	1.67	35.67
5 .....	1.0-1.45	1.70	35.70
1 .....	1.5-1.95	2.00	35.50
2 .....	2.0-2.45	1.75	34.75
28 .....	Average 0.54	1.29	35.86
II. Neutralized cream butter without butter culture			
5 .....	Below-0	0.40	35.20
12 .....	0 -0.45	0.33	35.17
11 .....	0.5-0.95	0.32	35.00
9 .....	1.0-1.45	0.89	34.67
8 .....	1.5-1.95	0.89	34.88
4 .....	2.0-2.45	0.25	35.13
2 .....	2.5 and over	1.00	34.50
51 .....	Average 1.01	0.51	34.97
III. Neutralized cream butter with butter culture			
2 .....	Below-0	0	33.50
1 .....	0 -0.45	0.50	36.00
2 .....	0.5-0.95	0	34.75
1 .....	1.0-1.45	1.00	35.00
1 .....	1.5-1.95	0.50	35.00
0 .....	2.0-2.45	1.00	33.50
1 .....	2.5 and over	1.00	33.50
8 .....	Average 1.06	0.38	34.25

week at 21° C. on the one hand and loss of score during storage or the score itself after storage on the other hand. A definite trend towards reduced keeping quality was noticed with high increases in the acid values of the butter, and especially the butterfat after storage for a week at 21° C. Table 2 brings out this relationship for the three types of butter. The trend was most pronounced with sweet cream butter, but perceptible also for neutralized cream butter without butter culture. The number of samples of neutralized cream butter with butter culture is too small to warrant conclusions.

Since the deterioration of the butter during storage, especially in the case of sweet cream butter, very often seemed to be in the nature of fat deterioration as judged from the criticisms of the judges, and since Frielinghaus (9) noted that the development of rancidity is accompanied by a significant increase in the ratio of butterfat acidity to butter acidity, the relationship of this ratio to the keeping quality of the samples was studied.

Table 3 indicates that the average acid ratios for each type of butter,

TABLE 3

*The acid ratio of salted butter as related to the loss in flavor score of such butter when held for 1 month at 0-5° C.*

Number of samples	The average acid ratio in the butter					
	Loss in score during 1 month at 0-5° C.	When fresh	After 1 week at 21° C.	Increase during week	After 1 month at 0-5° C.	Increase during month
I. Sweet cream butter						
9	2.0- 2.5	62.4	72.1	9.7	81.0	18.6
12	1.0- 1.5	70.8	67.7	- 3.1	74.9	4.1
6	0 - 0.5	71.8	66.7	- 5.1	74.0	2.2
1	- 1.0- - 0.5*	73.8	63.6	- 10.2	60.6	- 13.2
28	Average 1.29	68.4	68.8	0.4	76.3	7.9
II. Neutralized cream butter without butter culture						
2	2.0- 2.5	78.9	81.2	2.4	85.3	6.4
13	1.0- 1.5	71.3	76.8	5.5	83.8	11.9
29	0 - 0.5	66.8	69.0	2.2	72.1	3.4
7	- 1.0- - 0.5	64.8	71.0	6.1	74.5	9.9
51	Average 0.51	68.2	71.7	3.5	75.7	7.5
III. Neutralized cream butter with butter culture						
2	1.0- 1.5	83.7	93.9	10.3	93.6	10.0
4	0 - 0.5	67.3	68.6	1.3	73.9	10.3
2	- 1.0- - 0.5	60.6	55.4	- 5.3	67.3	10.0
8	Average 0.38	69.7	71.6	1.9	80.4	10.7

when fresh, after 1 week at 21° C., and after 1 month at 0-5° C. were generally larger as the losses in flavor score increased. The only exception was in the fresh sweet cream butter where higher average acid ratios were found associated with the best keeping butter. However, after a week and

after a month, this relationship was reversed so that a large increase in the average acid ratio was associated with large score losses while decreases in the average acid ratio were associated with small score losses. For instance, in the samples which lost 2.0 to 2.5 points in flavor score during storage, the butterfat acidity was on the average 62.4 per cent of the butter acidity in the fresh butter, and this percentage increased to 72.1 after 1 week at 21° C. and to 81.0 after 1 month at 0-5° C.. In the samples which lost little or even gained in score during storage, the average acid ratio decreased or increased only very slightly during storage. The smaller the loss in score was during storage the greater was the average decrease in acid ratio. Thus the keeping quality of sweet cream butter was increased when the acidity of the fat increased at a slower rate than the acidity of the butter. This relationship was not quite as apparent in the samples of neutralized cream butter but held true for the few samples of neutralized butter made with butter culture.

TABLE 4

*The change in the acid ratio of salted butter during storage at 21° C. and 0-5° C. as related to the keeping quality of such butter when held for 1 month at 0-5° C.*

Number of samples	Increase in acid ratio during 1 week at 21° C.		Loss in score during 1 month at 0-5° C.	Score after 1 month at 0-5° C.
	Range	Average		
I. Sweet cream butter				
10 .....	- 5.0 and less	- 11.80	0.90	36.25
5 .....	- 4.9 to - 0.1	- 2.36	0.90	35.80
4 .....	0 to 4.9	2.10	1.63	35.63
9 .....	5.0 and over	14.54	1.78	35.56
15 .....	- 24.2 to - 1.2	- 8.65	0.90	36.10
13 .....	0.2 to 39.1	10.72	1.73	35.58
II. Neutralized cream butter without butter culture				
16 .....	- 39.6 to - 1.7	- 10.81	0.59	35.19
35 .....	0.1 to 34.7	10.18	0.47	34.50
III. Neutralized cream butter with butter culture				
5 .....	- 9.4 to - 1.1	- 6.80	0.20	34.50
3 .....	4.8 to 29.8	16.37	0.67	34.50
	Increase in acid ratio during 1 month at 0-5° C.			
	range	average		
I. Sweet cream butter				
10 .....	- 20.3 to - 0.7	- 8.33	0.95	36.20
16 .....	1.1 to 42.7	19.34	1.56	35.69
II. Neutralized cream butter without butter culture				
15 .....	- 43.8 to - 0.9	- 7.70	0.20	35.27
29 .....	1.2 to 36.6	13.62	0.64	34.91

To show still more distinctly the effect of the change of the acid ratio on the keeping quality of salted butter table 4 is presented. It indicates that when the acid ratio in sweet cream butter decreased during 1 week at 21° C., the average loss in flavor score during storage at 0–5° C. for 1 month was only about half as great as when the ratio increased. Furthermore, the average score itself after storage was distinctly higher when the acid ratio decreased than when it increased. In the case of neutralized cream butter, the average loss in score under these conditions was nearly the same, although the actual score after storage averaged somewhat higher.

In the case of butter made from neutralized cream using butter culture, the average loss in score was smaller but the average final score was the same, when the acid ratio decreased, as compared with an acid ratio increase.

Considering the effect of the change in acid ratio on the keeping quality of individual samples, it was found that of the 9 samples of sweet cream butter which lost 2.0–2.5 points in score during storage 8 showed an increase in the acid ratios during 1 week at 21° C. and of 13 samples which lost 1 point or less in storage 10 samples showed a decrease in the acid ratio during the week at 21° C. Thus a knowledge of the change in the acid ratio seems to offer a valuable aid in foretelling keeping quality, at least for sweet cream butter. Although the acid ratio itself after 1 week at 21° C. is of some value for this purpose, it is not as significant as the change in ratio.

The average acid ratio for each type of butter studied was higher after storage than when fresh and higher after 1 month at 0–5° C. than after 1 week at 21° C. The figures are shown in table 3. Table 4 also brings out the relationship of the change in the acid ratio during a month at 0–5° C. to the keeping quality of the butter. Here an increase in the acid ratio is shown to be associated with a marked decrease in keeping quality of both sweet cream and neutralized cream butter.

A study of individual samples revealed that in 22 of the 26 sweet cream butter samples, 37 of the 44 neutralized samples and in 4 of the 5 cultured samples for which complete data were available, the acid ratio changed in the same direction during the week at 21° C. as during the month at 0–5° C.

#### DISCUSSION

It seems that no correlations exist between acid values and keeping quality which are definite enough to enable the prediction of keeping quality for every individual sample of butter. At the same time, certain trends were noticed which deserve attention when attempting to predict the keeping quality of salted butter, especially sweet cream butter. Furthermore, since the nature of butter deterioration may vary greatly, and since the accuracy of butter scoring standards is far from what is desirable, such

general trends, as were observed in this study, may have considerable significance.

On the basis of the observations made here on the acid values of butter and butterfat, it seems that the most valuable aid in the prediction of the keeping quality of sweet cream butter is the change, during 1 week at 21° C., in the percentage of the butter acidity which is closely associated with the butterfat. When this acid ratio increased during 1 week at 21° C. the loss in score during storage for 1 month at 0-5° C. was considerably greater, as a general rule, than when the ratio decreased. Acidity develops independently in butter and in butterfat during storage and it is the acidity increase of the fat in relation to the acidity increase of the butter taking place during storage, which appears to be a significant indicator of the keeping quality of butter when stored for 1 month at 0-5° C.

The acid ratio itself after 1 week at 21° C. also averaged higher in the butter of the poorer keeping qualities. Furthermore there was a definite trend toward reduced keeping quality in salted butter with increases in the butter acidity, and especially the butterfat acidity during 1 week at 21° C. However, the acid values themselves, either in the butter or in the butterfat, when fresh and after 1 week at 21° C. apparently are of less significance as an index of keeping quality.

#### SUMMARY

The acid content, calculated as lactic acid, and as determined in this investigation for 26 samples of sweet cream butter with pH values above 6.0 was 0.07-0.13 per cent when fresh, 0.072-0.145 per cent after 1 week at 21° C., and 0.079-0.131 per cent after 1 month at 0-5° C. It averaged 0.096 per cent when fresh, 0.109 per cent after 1 week at 21° C. and 0.103 per cent after 1 month at 0-5° C. For 51 samples of neutralized cream butter these averages were 0.122, 0.140 and 0.132 per cent, respectively.

High acid values of butter and butterfat before and after storage showed a slight tendency toward reduced keeping quality. High increases in the acid values after storage at 21° C. and 0-5° C. did show a fairly close correlation with reduced keeping quality of sweet cream and neutralized cream butter but especially of sweet cream butter.

An increase in the acid ratio (fat acidity: butter acidity) during 1 week at 21° C. and during 1 month at 0-5° C. seemed to be closely related to poor keeping quality, especially in the case of sweet cream butter. The average acid ratios were higher after 1 month at 0-5° C. than after 1 week at 21° C.

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# THE DENSITY AT 140° F. OF THE MATERIALS EXPRESSED AS FAT BY VARIOUS VOLUMETRIC TESTS OF CREAM\*

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The density of the material comprising the fat columns in the various volumetric tests has received very little attention. This is undoubtedly due to the fact that the procedures of the tests have usually been adjusted so that the results will agree with the official method. There has been some investigation as to the absolute accuracy of some of the rapid methods of testing dairy products for fat. The Babcock test bottles are calibrated for fat of a definite density at a definite temperature. Several modifications of the Babcock procedure have been proposed. It seems obvious that a modification using alcohol would give a fat column of a density different from that of a test using acid. Babcock (1) in devising his test bottle assumed the specific gravity of the fat to be 0.9000 at 120° F. He did not believe that a variation in temperature of reading from 110 to 150° F. would materially affect the results for milk although he recommended using the higher temperature. The difference for the 40 degree range was given as being less than 0.1 per cent for a 5 per cent milk test. In 1891 Farrington (2) obtained the greatest accuracy compared to gravimetric methods if the fat column in the Babcock milk test was read at 140° F.

The directions for testing cream were likewise lax in spite of the greater differences that were bound to occur. Webster (3) in 1904 reported the density of fat to be 0.9004 at 100° F., but by weighing a definite amount of fat into a test bottle he was able to account for all of it only by reading at 120° F. A little later Hunziker, Spitzer, Mills and Crane (4) insisted that the correct reading temperature was 135° F. for that was the temperature at which the fat had a specific gravity of 0.9000. Then Ross and McInerney (5) recommended a reading temperature between 140° and 150° F., obtaining better checks with ether extraction at 150° F., but for milk they reported that there was no difference by reading at either 100° or 146° F. Bailey (6) disagreed with all previous investigators when he reported that all density measurements at Iowa State College showed the density of butterfat to be about 0.8974 at 120° F. He states that the density varies by 0.00038 per degree F. This gives a density of 0.9000 at about 113° F. Hepburn (7) used a temperature of 125 to 130° F. for his modified Babcock test for butter. Dahlberg (8) found the specific gravity to be 0.8943 at 132° F., however he

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read at a temperature of 132° F. and reported that the Babcock bottle was calibrated for 0.9000 at 135° F. Tracy and Overman (9) recommended reading the test at the now commonly accepted temperature of 135–140° F. Some other investigators gave the following specific gravity values for butterfat: Storch (10) 0.9335 at 15° C. (59° F.), Rahn (11) 0.89857–0.89729 at 50° C. (122° F.), and Koestler (12) 0.9355–0.9448 at 15° C. (59° F.).

The data reported by various investigators on the density of butterfat may be summarized as follows:

Investigator	Density of fat reported	Read Babcock test at
Babcock (1)	0.9000 at 120° F.	110 to 150° F.
Farrington (2)		140° F.
Webster (3)	0.9004 at 100° F.	120° F.
Hunziker and coworkers (4)	0.9000 at 135° F.	135° F.
Ross and McInerney (5)		140 to 150° F.
Bailey (6)	0.8974 at 120° F. 0.9000 at 113° F.	130° F.
Hepburn (7)		125 to 130° F.
Dahlberg (8)	0.8943 at 132° F.	132° F.
Tracy and Overman (9)		135 to 140° F.
Storch (10)	0.9335 at 15° C. (59° F.)	
Rahn (11)	0.89857 to 0.89729 at 50° C. (122° F.)	
Koestler (12)	0.9355 to 0.9448 at 15° C. (59° F.)	

Variations in the composition of butterfat due to feed and other factors may partially explain such conflicting results.

#### EXPERIMENTAL

Cream with a fat content of about 35 per cent obtained from mixed herd milk during the late fall and winter was tested by the Mojonnier method, by the Babcock method and by the following modifications of the Babcock method: the Minnesota 202, the Minnesota Nafis, the N-butyl alcohol, and the amyl alcohol. The Minnesota 202 refers to the original Minnesota Babcock Test reagent and the Minnesota Nafis to the reagent as sold by the Nafis Company. In the N-butyl alcohol test 1 ml. of N-butyl alcohol was used in addition to sulfuric acid to 9 gr. of cream. In the amyl alcohol test 2 ml. of amyl alcohol and about 17 ml. of sulfuric acid was used to 9 gr. of cream to give about the same proportion as in the Gerber test.

Picnometers having a volume of about 1 ml. were calibrated with mercury and used in determining the density of the material read as fat in the various tests. As check samples a small portion of the cream was churned and the resulting butter melted and filtered to obtain practically pure butterfat. The results of these determinations are summarized in tables 1 and 2. A marked variation was found to exist in the density of the fatty materials from the various tests when measured at 140° F. At this temperature the pure

butterfat had an average density of 0.89169; the Babcock fat column, 0.89512; the Minnesota Nafis fat column, 0.89085; and the Minnesota 202 fat column, 0.88398. Calculating the per cent overreading, which theoretically would occur due to the density not being exactly 0.9000 at 140° F., gave an error of 0.932 per cent that would be encountered even if the fat columns were pure fat. Similarly the calculated error due to the specific gravity of the material read as fat at 140° F. amounted to 0.545 per cent for the Babcock, 0.825 per cent for the N-butyl alcohol, 0.966 per cent for the amyl alcohol, 1.027 per cent for the Minnesota Nafis, and 1.812 per cent for the Minnesota 202 test.

TABLE 1

*The density at 60° C. (140° F.) of the materials read as fat with the various tests of cream*

Determi-nation date	Filtered butterfat	Mojonnier	Babcock	Nafis	202	N-butyl	Amyl
10/7/35		.89410	.89359	.89036	.88406		.89065
10/21/35	.89261 .89400	.89252 .89410	.89644	.89185	.88299 .88456	.89056 .89288	.89136 .89232
1/18/36	.89199 .88998	.89341 .89322	.89403 .89643	.88994 .89148	.88433 .88400	.89368 .89344	.89029 .89241
Mean	.89169	.89347	.89512	.89085	.88398	.89264	.89139

Bailey (6) reported that the density of butterfat varied by 0.00038 per degree Fahrenheit. Using this value it was calculated that the fatty materials studied would have an approximate density of 0.9000 at 118° F. for pure butterfat, at 127° F. for the Babcock test fat column, 116° F. for the Minnesota Nafis test, 72° F. for the Minnesota 202 test, 117° F. for the amyl alcohol test and 121° F. for the N-butyl alcohol test. The effect of impurities in the fat columns of the various tests would probably change the above calculated values.

The higher density of the Babcock test fat columns over pure butterfat was probably due to the inclusion of water, while in the case of the 202 test

TABLE 2

*The calculated error due to reading the various fat columns at 140° F., and the calculated temperature at which the fat columns would have a density of 0.9000*

Testing method	Error at 140° F.	Temperature at which* density would be approx. 0.9000
Pure butterfat .....	per cent	°F.
Babcock .....	+ 0.932	118
Nafis .....	+ 0.545	127
202 .....	+ 1.027	116
N-butyl .....	+ 1.812	72
Amyl .....	+ 0.825	121
	+ 0.966	117

\* Assuming the density of fat varies by 0.00038 per degree F. (6).

the lower density was probably due to alcohol. The presence of these impurities in the fat column introduces a fundamental error.

The results here reported indicate that interesting results may be obtained by conducting further work to determine the proper temperature at which to read the various tests.

#### SUMMARY

1. The density of the materials read as fat with the various volumetric tests was less than 0.9000 at 140° F.
2. The error in the volumetric tests due to variation in the density at 140° F. of the materials read as fat was the least with the Babcock test (about 0.55 per cent) followed in order by the N-butyl alcohol, the amyl alcohol, the Minnesota Nafis and the Minnesota 202 test (over 1.8 per cent).

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## REDUCTION OF CURD TENSION OF MILK BY THE ADDITION OF SODIUM SALTS

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For a good many years some formulae used in infant feeding have specified the addition to the milk of salts, such as sodium citrate. Bosworth and VanSlyke (1) in 1914 in commenting on the earlier work of Talbot done in 1905 in which he showed normal milk to form large curd particles in the human stomach, stated, "These lumps of curd may pass practically unchanged through the entire intestinal canal, causing mechanical irritation, which often results in serious interference with the process of normal digestion. Empirical practice has shown that this abnormal curdling of milk may, to some extent, be modified or controlled by the addition of sodium citrate at the rate of 1 or 2 grains per ounce of milk." Bosworth and VanSlyke explained the failure of milk containing sodium citrate to curdle upon the addition of rennet as being due to the formation of calcium sodium paracaseinate which is quite soluble. While other methods have been employed to reduce curd tension in milk, such as high-heat treatment, base exchange, addition of enzymes, and homogenization, so far as the authors are aware no commercial operations are practiced which involve the direct addition of sodium salts, even though the work of Bosworth and VanSlyke, done 25 years ago, clearly indicated the possibilities of such a practice. Schwartz, Jones, Mack, and Vance (2) reported at the 1939 annual meeting of the American Dairy Science Association upon the use of sodium metaphosphate for the preparation of soft-curd milk.

### EXPERIMENTAL

The method of measuring curd tension in this study was the one recommended by the committee on curd tension measurement (of the American Dairy Science Association) which reported at the 1938 meeting in Columbus, Ohio. This method involves the use of a pepsin hydrochloric acid coagulant. The curdometer used was one manufactured by the Submarine Signal Company. The milk was supplied by the University herd.

While it is recognized that there are several salts of sodium that might be successfully used to soften the curd of milk, this study was limited to a consideration of sodium citrate, sodium pyrophosphate and sodium hexametaphosphate.

### USE OF SODIUM CITRATE

To study the use of sodium citrate as a softener of the curd of milk, varying amounts of the salt were added to raw milk which was heated to

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143° F. and held for 30 minutes. Some of the milk was then homogenized at 2500 pounds pressure. Data were secured on the curd tension values of the raw, pasteurized, and homogenized milk, the flavor of the milk and the creaming qualities of the pasteurized milk.

TABLE 1  
*Effect of sodium citrate upon curd tension of milk*

Sodium-citrate	Curd tension		
	Raw	Pasteurized	Homogenized
per cent	grams	grams	grams
None	46	43	13
.10	27	30	14
.15	18	23	13
.20	0	1	3
.25	0	0	0

It is interesting to note that the curd tension of the milk to which sodium citrate had been added was higher after pasteurization. It will also be observed that up until zero curd tension was obtained the proportional decrease in curd tension caused by the sodium citrate was greater in the case of both the raw and pasteurized milk than it was in the case of the homogenized milk.

The titratable acidity of all the milks to which sodium citrate was added was only slightly affected, being reduced from 0.15 to 0.145 per cent. The flavor of the milks was made salty by the addition of sodium citrate but peculiarly the salty flavor was less noticeable in the case of the homogenized milk.

That the sodium citrate in the amounts used had no appreciable effect upon the creaming qualities of the milk to which it was added is shown by the data in table 2.

TABLE 2  
*Effect of sodium citrate upon creaming of pasteurized milk*

Sodium citrate added	Cream volume				
	2 hrs.	5 hrs.	8 hrs.	24 hrs.	48 hrs.
per cent	per cent	per cent	per cent	per cent	per cent
None	15 -15	17 -16.5	17 -16	17 -16	14.5 -15
.10	14 -13.5	16 -16	16.5 -15	18 -17	15.5 -16.5
.15	11 -12	11 -12	.....	14 -15	15.5 -15.5
.20	14.5 -14.5	16 -16	17 -16.5	18 -16.5	17 -16
.25	11 -12	14 -13	14 -14	15.5 -15.5	15 -15.5

Upon adding 2 parts per million of copper to the pasteurized experimental milks it was found that there was less tallowy flavor developed in the milks to which the sodium citrate was added.

## USE OF SODIUM PYROPHOSPHATE

Having determined that a sodium salt such as sodium citrate would produce an appreciable reduction in the curd tension of milk an attempt was then made to find a sodium salt that would produce similar effects upon the curd tension of milk with less effect upon the flavor. For this purpose sodium pyrophosphate, a salt commonly used as an emulsifier in the manufacture of process cheese, was first tried. Table 3 shows the relative effects which the same amounts of sodium citrate and sodium pyrophosphate had upon the curd tension of milk. The greater effect of the pyrophosphate is clearly shown. The effect of heat in slowing up the effect of this salt, and its proportionally lesser effect upon the curd tension of homogenized milk is further indicated by the results in table 3.

TABLE 3

*Relative effect of 0.15 per cent sodium citrate and 0.15 per cent sodium pyrophosphate upon the curd tension of milk\**

Sample	Curd tension	
	Sodium citrate	Sodium pyrophosphate
	grams	grams
1. Salt added to pasteurized milk before heating to 143° F. for 10 minutes .....	27	4
2. Salt added to pasteurized milk not heated ..	18.5	0
3. Same as 1 except homogenized after pasteurization but before addition of salt .....	15.5	4
4. Same as 3 except not reheated after addition of salt .....	6	2.5

\* Curd tension of control pasteurized—48 grams.

Curd tension of control pasteurized and homogenized—11 grams.

An additional comparison of sodium citrate and sodium pyrophosphate is given in table 4. In this experiment an attempt was made to combine the

TABLE 4

*Reducing curd tension by combining the effects of high-heat treatment and the addition of a sodium salt*

Heat treatment given milk	Curd tension		
	Control	0.15 per cent Na citrate	0.10 per cent Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub>
	grams	grams	grams
143° - 30 minutes	54	44	30
170° - 0 minutes	49	24	16
" - 2 "	39*	18*	14.5
" 4 "	34	8	8.5
" 6 "	30	7	3.0*
" 8 "	30	4.5	3
" 10 "	24	2.5	3

\* Indicates point where cooked flavor became sufficiently strong to be noticeable.

effects of high-heat treatment and the sodium salts in producing soft-curd milk. For this purpose the milk was heated rapidly to 170° F. and a sample immediately taken and other samples taken every 2 minutes for 10 minutes. The temperature of 170° F. was arbitrarily selected as one representing the upper limit to which milk could be heated without acquiring an objectionable cooked flavor. The amounts of sodium salts used were such as to produce an appreciable effect upon the curd without too noticeable an effect upon the flavor of the milk. It is interesting to note that less cooked flavor was detectable in the milk to which the pyrophosphate was added. It would seem that where lack of cream line is not a factor, a satisfactory low-curd tension milk could be produced by heating the milk to 170° F. for 4 to 5 minutes, cooling rapidly and then adding the sodium salt.

That this treatment would not be particularly advantageous in the case of homogenized milk is shown by table 5.

TABLE I

## *Reducing curd tension of homogenized milk by combining the effects of heat treatment and the addition of 0.1 sodium pyrophosphate*

## USE OF HEXAMETAPHOSPHATE TO REDUCE CURD TENSION IN MILK

Previous mention has been made of the use of sodium metaphosphate by Schwartz *et al.* to produce soft-curd milk. However, since this salt hydrolyzes rapidly to form dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ) which is rather inactive at the pH of milk, it was thought best to use the hexametaphosphate in our studies. This salt hydrolyzes rather rapidly in the presence of heat to form dihydrogen phosphate. A commercial form of hexametaphosphate (Calgon) on the market as a water softener was also studied. This product contains a small amount of soda ash to reduce hydrolysis.

In the preliminary trials the hexametaphosphate was found to be more effective than pyrophosphate in reducing curd tension. As would be expected, heating the milk with the salt resulted in a higher curd tension than that obtained by adding the salt to the cold milk. Undoubtedly this was due to the hydrolysis of a certain portion of the hexametaphosphate to

form the less active dihydrogen phosphate. A comparison of the four salts studied is given in table 6.

TABLE 6

*Comparison of different sodium salts in their effect upon curd tension and flavor of milk*

Sample	Curd tension				Flavor (after 24 hrs.)
	Fresh	24 hrs.	48 hrs.	72 hrs.	
	grams	grams	grams	grams	
1. Control .....	51	51	50	48	Satisfactory
2. .05% Calgon .....	38	35	27	28	Slight slickness and burning
3. .075% " .....	8.5	5.5	0	0	" "
4. .10% " .....	0	1	0	0	" "
5. .05% hexametaphosphate .....	36.5	35	28	26	" "
6. .075% " .....	5.5	3	0	3	" "
7. .10% " .....	0	0	0	0	" "
8. .10% pyrophosphate .....	33	33	29	27	" "
9. .15% " .....	12	13	12	13	" "
10. .20% " .....	5.5	4.5	1.5	4	" "
11. .10% sodium citrate .....	38	40	33	31	Slightly salty
12. .15% " .....	31	31	22	21	" "
13. .20% " .....	12	12	3	0	" "

The titratable acidity of the milk was practically unaffected by the salts. Cream-line measurements of each sample were made periodically up to 48 hours and, with the exception of the sample containing 0.20 per cent pyrophosphate, there were no significant differences. The sample referred to was noticeably more viscous than the others and failed to show any cream line even after 48 hours.

#### DISCUSSION

A permanent reduction in the curd tension of milk can be obtained by the addition of sodium citrate, sodium pyrophosphate, or sodium hexametaphosphate. The amounts required will vary with the milk and the results desired. When using milk of approximately 50 grams curd tension, 0.075 per cent of the sodium hexametaphosphate, 0.15 per cent of the sodium pyrophosphate or 0.20 per cent of the sodium citrate are necessary to reduce the curd tension to 0-10 grams.

The flavor of the milk is affected to some extent by the addition of these salts. The citrate produces a salty flavor while the phosphate salts produce a slight burning sensation at the edges and tip of the tongue. All of the milks after treatment have a slight slickness of body. It was found possible to produce a superior flavored product by combining equal portions of samples 6 and 12 (table 6) suggesting that the flavor effect of the two salts might be minimized by using an amount of each salt necessary to produce in their combined effect the desired curd tension. This was done in one case by using 0.0375 per cent of the hexametaphosphate and 0.075 per cent of the sodium citrate.

A lower curd tension results when the sodium salts are added after pasteurization to the cooled milk. After adding the salt, the curd tension continues to decrease, reaching an equilibrium after about 48 hours.

Normal creaming of the milk is not affected until sufficient salt is added to change the viscosity. The titratable acidity of the milk remains practically unchanged after treatment.

The desirability of using sodium salts to lower the curd tension of milk remains to be established. Before the procedure is used commercially it should be proven by sufficient clinical evidence that milk with a curd tension lowered by the application of sodium salts has superior nutritional qualities. It also will be necessary to have official sanction from the health officials since such additions to milk ordinarily would be considered adulteration.

#### SUMMARY

A method has been presented for producing milk with reduced curd tension by the addition of certain sodium salts. It is indicated that there is need for further nutritional study of the product before recommending adoption of the method by the industry.

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## POTENTIOMETRIC STUDIES WITH RESAZURIN AND METHYLENE BLUE IN MILK<sup>1</sup>

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Comparative studies with resazurin and methylene blue as indicators of the bacteriological quality of milk have been carried on in these laboratories for several years. It has been found that the resazurin "one hour" test is less reliable than the methylene blue test in that milks containing large numbers of dormant organisms frequently fail to cause any significant change in color during this period (8). Furthermore, seasonal and breed differences in pigmentation complicate color comparisons, while some workers have difficulty in distinguishing the intermediate shades of color. When, however, incubation is continued to the pink stage, there is an excellent correlation between reduction times for resazurin and methylene blue, the ratio being approximately 3:4<sup>2</sup> (fig. 1). Calculation of the coefficients of correlation between reduction times and Breed counts of individual cells for 369 samples of market milk yielded the following values:

Resazurin:  $r = -0.711 \pm 0.017$

Methylene blue:  $r = -0.651 \pm 0.020$

Consequently, this modified resazurin test, using the pink end-point, may be considered at least as accurate as the methylene blue test as an index of the bacterial content of milk at the time the test is begun.

We have been interested in discovering the reason for the significantly shorter time required for the reduction of resazurin to the pink stage. Resazurin is slightly more electropositive than methylene blue (9). However, the shape of the time-potential curve ordinarily obtained with either plain milk or milk + methylene blue is such that it is difficult to believe that the slight difference  $E'$  values for these two dyes is sufficient to account for the marked shortening in reduction time<sup>3</sup> indicated in figure 1. The only other explanation that suggests itself is that resazurin may change the shape of the time-potential curve so that the zone of reduction will be reached somewhat earlier than with milk + methylene blue or plain milk. Studies were

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<sup>2</sup> In these studies, the errors resulting from undisturbed creaming (3, 7, 10, 13) were minimized by inversion of tubes at regular intervals until incipient reduction was noted. The wider ratio (1:2) reported by Collins *et al.* (1) may be due to their incubating tubes undisturbed, or to end-point differences.

<sup>3</sup> Reduction time for resazurin represents the time taken to change to a full pink color; for methylene blue, the time required to decolorize at least 90 per cent of the column of milk.

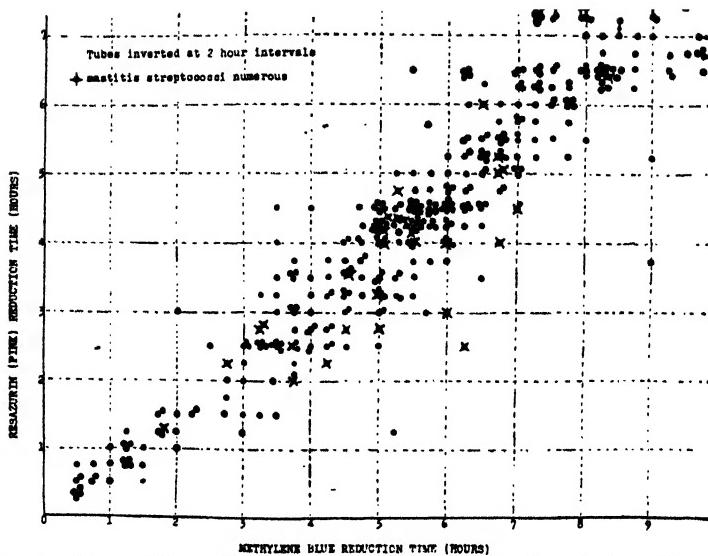


FIG. 1. Comparative reduction times with resazurin and methylene blue for 369 market milks.

therefore undertaken to shed some light on this and other points in connection with these dye reduction tests.

#### EXPERIMENTAL

The electrode vessels consisted of 1" x 8" test tubes, closed with 3-hole rubber stoppers through which passed two spiral platinum electrodes and a tube containing saturated KCl. The latter was narrowed at the base and crimped onto asbestos fiber to prevent the rapid leakage of the solution. The top of the tube was flared to facilitate insertion of a KCl-agar bridge.<sup>4</sup> Six tubes were set up at one time, connections being made to a Beckman Model G vacuum tube potentiometer through a specially constructed 12-way switch.

After autoclaving while filled with distilled water, the tubes were emptied and 2.5 ml. of dye solution<sup>5</sup> added. Twenty-five ml. of milk were then introduced into each tube and the tubes set in a water-bath at 37° C. In general, half of the tubes were carefully inverted at hourly intervals, the remainder being disturbed as little as possible.

Although at times marked variability characterized the readings from

<sup>4</sup> This type of connection was devised and constructed by Mr. G. B. Landerkin, M.Sc., of this Division.

<sup>5</sup> Resazurin (EK 2106) to give a concentration of 1:200,000 in milk. Methylene blue thiocyanate (Na. t.1) to give a concentration of 1:300,000 in milk. An equivalent quantity of sterile distilled water was added to control tubes.

duplicate electrodes, it was soon evident that the shape of curve for milk plus resazurin differed from that for plain milk or milk plus methylene blue. The curves shown in figures 2 and 3 are representative of those from a wide

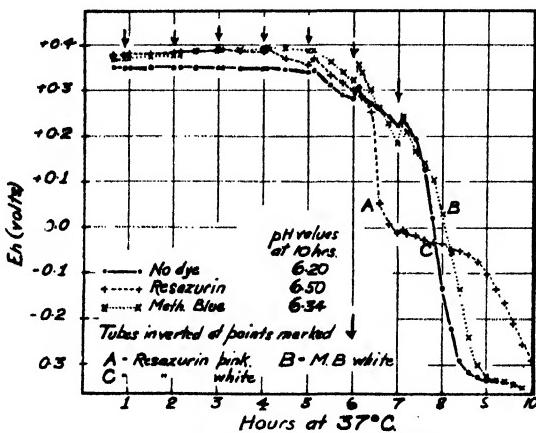


FIG. 2. Time-potential curves for milk, milk plus resazurin and milk plus methylene blue at  $37^{\circ}\text{C}$ .

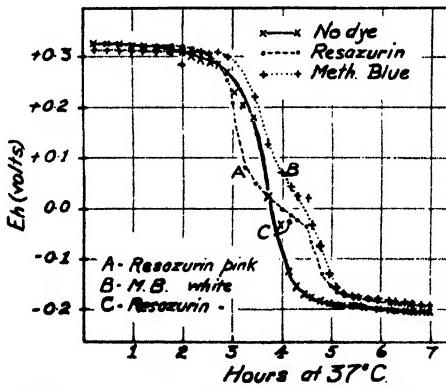


FIG. 3. Time-potential curves for milk, milk plus resazurin and milk plus methylene blue at  $37^{\circ}\text{C}$ .

variety of milks. It will be noted that the curve for milk plus resazurin shows a sharper initial drop in Eh than do those for milk plus methylene blue or plain milk. This drop is followed by a flattening of the curve, starting at a point near that at which the full pink color appears. Later the curve again declines, eventually reaching the same final Eh level as the others.

This flattening of the curve after reaching the pink stage suggests that resazurin exerts a stronger poising action than does the older dye. Time-potential curves obtained with portions of the same lot of milk containing

one-half strength, full strength and double strength resazurin are shown in figure 4. (In this graph the scale of the X axis has been doubled in order

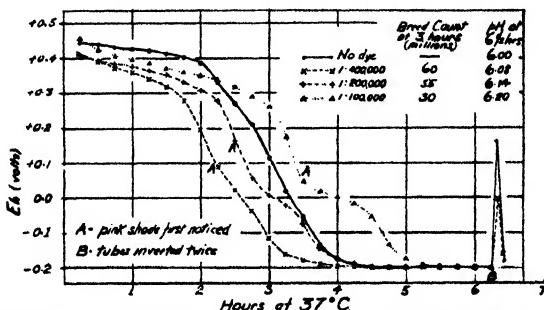


FIG. 4. Influence of resazurin concentration upon time-potential curves at 37° C.

that differences may be more readily noted. The points at which the appearance of the pink color were noted are only approximations.) These curves indicate: 1, that the degree of flattening of the curve is proportional to the dye concentration, and 2, that the double strength dye exerts a definite bacteriostatic influence. The latter is also borne out by the pH values at 6½ hours and by the Breed count of individual organisms made from a parallel set of tubes at the 3rd hour.

Turning now to the influence of inversion upon the time-potential curve (figs. 2 and 5) it will be noted that it causes little or no change during the

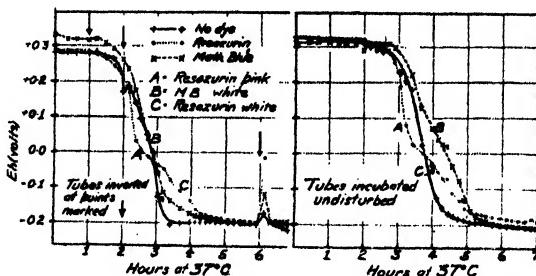


FIG. 5. Influence of mixing upon time-potential curves.

first period when the curve is approximately horizontal. As the Eh drops, however, the effect of inversion becomes more pronounced. However, the Eh changes are of a transient nature and have little influence upon the general trend of the curve. On the other hand, dispersion of the cream layer and accompanying bacteria frequently results in much earlier reduction of both resazurin and methylene blue (fig. 5). That this may be due to more active growth is indicated by the higher hydrogen ion concentration in mixed than in unmixed tubes at the end of the run (table 1). The data

TABLE 1

*Influence of hourly inversion of tubes upon final pH values\**

Date	Hours in-cubated at 37° C.	Plain milk		Milk + meth. blue		Milk + resazurin	
		A	B	A	B	A	B
Nov. 22.....	9½	5.97	5.51	6.02	5.68	6.05	5.80
" 24.....	9½	5.85	5.52	6.00	5.65	6.00	5.70
" 25.....	10½	6.00	5.81	6.21	6.00	.....	.....
Dec. 2.....	9½	6.18	6.00	6.28	6.10	6.31	6.18

\* Determined with glass electrode.

A = Tubes incubated undisturbed.

B = Tubes inverted hourly until incipient reduction noted.

in table 1 indicate that at the concentrations employed, resazurin exerts a slightly greater bacteriostatic influence than does methylene blue. Experience with several hundred samples of market milk has failed to reveal any undesirable effect attributable to inversion of tubes at intervals of 1 to 2 hours during incubation. In passing, attention may be called to the unusually low final Eh levels reached in certain samples (figs. 2 and 6). Some of

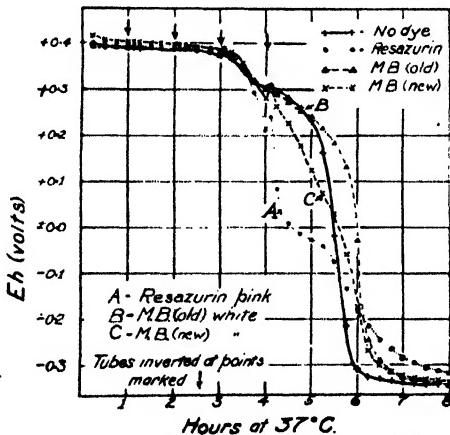


FIG. 6. Influence of mixing upon time-potential curves.

these were taken from a 3,000 lb. vat, others from the weigh-tank (individual shippers). Wilson (13) reports similar low levels in pure culture studies with *Esch. coli* and *Aerobacter aerogenes*, but we have seen none recorded for mixed milks.

It is now generally realized that certain organisms show much less active reduction of potential than do the majority of bacteria (4, 5, 13). In at least one area, milks containing numerous thermoduric organisms have failed to reduce methylene blue within 8 hours when incubated undisturbed. In view of its higher Eh range, and of the earlier downward bend of the

curve, it seemed likely that resazurin should prove useful in detecting such milks.

Through the courtesy of Dr. E. H. Parfitt, representative cultures of these thermouduric organisms have been tested out with both dyes in the ordinary reduction test as well as potentiometrically. Only a gradual decline in Eh has been observed even when the direct microscopic count at the start showed 5,000,000 individual bacteria per ml.<sup>6</sup> The color changes in the dye-milk mixtures are correspondingly gradual. There is usually partial reduction of methylene blue by the time resazurin reaches the pink stage. However, a trace of blue color remains in the body of the tube for some hours.<sup>7</sup> Data presented in table 2 suggests that if the pink stage of resazurin is taken as the end-point, this test will grade these milks somewhat more satisfactorily than will methylene blue. Since the optimum temperature for these organisms is below 37° C., growth is encouraged by preliminary incubation at 12.8° C. for 18 hours, as previously advocated (6), and the reduction time significantly shortened.

Substitution of methylene blue thiocyanate for the chloride, and increase in dye concentration to 1: 300,000 have been recommended (12). The latter modification lengthens reduction time, Thornton and Sandin (12) reporting an increase of 30 minutes, Johns (7) 20 per cent and Frayer (3) 100 per cent increase. Although Thornton *et al.* (11) report reduction over the same approximate Eh range for both dye concentrations, our studies indicate that this lengthening of reduction time is due to the increase in dye concentration, as suggested by Fay and Aikin (2). Despite the obvious difficulty of determining the exact potential at which decolorization occurs, we have invariably noted visual reduction at a definitely higher level with the weaker concentration. In several instances the average value from duplicate electrodes has been almost 200 mv. more positive. The points noted in figure 6 are fairly representative of a variety of milks we have studied potentiometrically.

#### SUMMARY

Resazurin reduces to the pink stage in approximately three-fourths of the time required for methylene blue to decolorize. Potentiometric studies indicate that this shortening of reduction time is mainly attributable to the change in shape of the time-potential curve when resazurin is present.

Hourly inversion of tubes during incubation generally shortens the reduction time of good milks considerably. The incorporation of oxygen by this practice has little or no effect upon the time-potential curve during the early stages, and only transient effect later.

<sup>6</sup> This may be attributed to the slow growth rate at 37° C. When incubated at room temperature, growth is more rapid and the Eh curve shows a steeper decline.

<sup>7</sup> With both dyes a narrow zone of fairly intense color may remain at the surface for hours after the resazurin pink stage has been reached.

TABLE 2  
Comparison of resazurin and methylene blue in evaluating milks containing weakly reducing organisms

Cult. No.	Resazurin color number* after (hours)						Reduction time (hours)			Initial Breed count individual cells per ml. (thousands)
	1	2	3	4	5	6	7	8	9	
1	2	6	8	8	8	13	20	7 <sup>†</sup>	>9	17.5
2	14	23	24	12	16	16	16	11 <sup>†</sup>	2	29,400
4	6	10	12	24	24	24	24	4	9	22,600
5	2	22	22	20	20	24	24	11 <sup>†</sup>	2 <sup>†</sup>	4,670
8	2	16	16	20	24	24	24	2	3 <sup>†</sup>	18,200
1	1	1	2	2	2	2	4	8	9	2,540
2	2	2	4	5	6	6	14	21	5 <sup>†</sup>	2,860
4	5	9	11	12	15	15	17	17	5 <sup>†</sup>	9,020
5	1	2	2	2	2	2	8	13	7 <sup>†</sup>	>9
8	1	3	3	4	4	6	13	19	6 <sup>†</sup>	2,220
4	4	12	15	17	18	19	21	23	3 <sup>†</sup>	2,960
4	8	12	16	16	16	20	20	24	3	18,600
									8 <sup>†</sup>	38,100

\* Color numbers range from initial full color (0) to pink (16) and to white (24).

† Pink taken as end-point of reduction.

Resazurin is more useful than methylene blue in detecting milks containing large numbers of weakly reducing organisms.

The lengthening of reduction time when the concentration of methylene blue is increased from 1:700,000 to 1:300,000 appears to be attributable to a downward shift in the Eh zone at which the dye decolorizes.

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## EFFECT OF LACTIC ACID ON THE HYDROLYSIS OF FAT IN CREAM BY PURE CULTURES OF LIPOLYTIC MICRO-ORGANISMS<sup>1</sup>

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It has been reported that the development of lactic acid in milk or cream has a restraining influence on the growth of undesirable types of micro-organisms. To study this problem, sterilized cream was inoculated at approximately the same time with a butter culture and a culture of a common lipolytic micro-organism. Lipolytic molds, yeasts and bacteria were used in these trials and included *O. lactis*, *Myc. lipolytica*, *Ach. lipolyticum*, *Alc. lipolyticus* and *Ps. fluorescens*. After inoculation, the lots of cream were held at 21° C. The acidity and flavor of the cream and acid number of the fat were determined after 2, 4 and 6 days.

After 6 days incubation at 21° C. (table 1) the fat in the sample inoculated only with the mold had an acid number of 40.4 while the sample inoculated with butter culture organisms as well as *O. lactis* had an acid number of 10.4. It is evident that the growth of butter culture organisms with the resultant formation of lactic acid inhibited the lipolytic activity of *O. lactis* in cream.

After 6 days incubation the sample containing only *Myc. lipolytica* had a fat acid number of 37.9 while the acid number of the fat in the sample inoculated with butter culture organisms as well as the yeast was 47.9. This indicates that the lactic organisms growing in the cream stimulated increased lipolytic action by the yeasts.

After 6 days incubation, the acid numbers of the fat of cream inoculated with cultures of lipolytic bacteria, with and without butter cultures respectively, were as follows: *Ach. lipolyticum* 2.5 and 8.3; *Alc. lipolyticus* 1.6 and 2.5 and *Ps. fluorescens* 2.5 and 4.4. All three species of lipolytic bacteria used in this study were definitely inhibited in their activity by the growth of butter culture organisms.

The question arose as to whether this inhibition was simply the result of the formation of lactic acid in the cream or whether the presence of growing butter culture organisms might have exerted some influence on the oxygen demands or other growth needs of the lipolytic organisms. In order to determine this point, lactic acid was sterilized and added to sterilized cream in such amounts that samples of the same lot of cream were obtained, varying in reaction from sweet in the check sample to very sour in the acidulated samples. These samples of cream were inoculated with various lipolytic

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<sup>1</sup> From a portion of a thesis presented to the Graduate School of Iowa State College in partial fulfillment of the requirements for the Ph.D. degree.

TABLE 1

*Effect of the growth of butter culture organisms in cream on the growth of lipolytic organisms*

Days held at 21° C.	Butter culture added			No butter culture added		
	Per cent acidity	Acid number	Flavor of cream	Per cent acidity	Acid number	Flavor of cream
<i>O. lactis</i>						
2	0.86	10.9	acid, rancid	0.17	12.5	fruity
4	.81	6.5	acid, rancid	.22	24.8	rancid, fruity
6	.77	10.4	acid, rancid	.24	40.4	rancid, fruity
<i>Myc. lipolytica</i>						
2	.86	2.7	rancid	.23	8.8	rancid
4	1.04	20.1	bitter, rancid	.38	20.1	rancid, bitter
6	.98	47.9	bitter, rancid	.38	37.9	rancid, bitter
<i>Ach. lipolyticum</i>						
2	.76	1.3	acid, old	.26	2.4	old
4	.95	2.1	acid, old	.31	4.5	acid, rancid
6	.96	2.5	acid, old	.37	8.3	rancid
<i>Alc. lipolyticus</i>						
2	.81	.5	acid	.19	.8	good
4	.87	1.0	acid	.23	2.4	old
6	.88	1.6	acid	.23	2.5	sl. rancid
<i>Ps. fluorescens</i>						
2	.88	1.4	acid, unclean	.19	1.4	rancid
4	.92	2.0	acid, unclean	.27	3.5	rancid, putrid
6	.96	2.5	acid, old	.27	4.4	rancid, putrid

micro-organisms. After incubating for 6 days at 21° C., the samples were churned and the acid numbers of the fat determined. As a check on the effect of the acid on the acid number of the fat, a series of acidulated but uninoculated samples was held for the same period as the inoculated samples and the acid numbers of the fat determined.

As shown in table 2, the uninoculated cream ranged in titratable acidity from 0.18 per cent (check sample) to 2.08 per cent in the sample receiving the largest portion of added lactic acid. No differences were observed in the acid numbers of the fat of this entire series of cream samples after incubation. These data indicate that lactic acid, even in concentrations greater than normally formed in cream, did not cause an increase in the acid number of fat.

*O. lactis* when inoculated into sweet or moderately sour cream increased the titratable acidity appreciably probably due to the liberation of acids from the fat. As the amount of added lactic acid was increased the action of the molds on the fat decreased. The samples of cream having titratable acidities of less than approximately 0.50 per cent when inoculated, showed

increases in titratable acidity due to the growth of the mold; samples having titratable acidities over approximately 0.50 per cent when inoculated, showed decreases. In general, the lower the titratable acidity of the cream when inoculated with *O. lactis*, the higher the acid number of the fat became, due to mold growth. From the data (table 2) it may be seen however that marked increases in the acid number of the fat were observed even when the mold was inoculated into cream having titratable acidities in excess of 1.0 per cent.

TABLE 2

*Effect of the addition of lactic acid on the growth of lipolytic micro-organisms in cream*

Lot	Cream acidulated—not inoculated		Cream acidulated and incubated 6 days at 21° C. after inoculation with					
			<i>O. lactis</i>		<i>Myc. lipolytica</i>		<i>Ach. lipolyticum</i>	
	Per cent acidity	Acid number of fat	Per cent acidity	Acid number of fat	Per cent acidity	Acid number of fat	Per cent acidity	Acid number of fat
1	0.18	0.5	0.46	26.1	0.60	-	0.41	11.3
2	.24	.7	.29	19.6	.46	-	.41	8.9
3	.41	.5	.45	15.8	.52	27.8	.49	7.8
4	.52	.7	.45	18.4	.60	21.8	.55	7.8
5	.71	.5	.55	14.0	.74	21.4	.66	5.0
6	.97	.5	.60	8.5	.85	13.7	.88	6.4
7	1.34	.5	.86	4.7	1.12	12.8	1.36	1.0
8	2.08	.6	1.34	3.7	1.72	3.1	2.17	.9

*Myc. lipolytica* when inoculated into samples of cream having varying acidities due to added lactic acid apparently grew luxuriantly even in the samples having very high titratable acidities. In table 2 it may be observed that a marked increase in the acid number of the fat occurred when cream having an acidity of 2.08 per cent was inoculated with this organism. In the cream having acidities above approximately 0.70 per cent, the organisms apparently utilized the acid in their growth since decreases in titratable acidities were observed in these samples.

*Ach. lipolyticum* formed some acid when growing in cream and in some of the trials was able to hydrolyze fat when inoculated into cream having a titratable acidity of about 1.0 per cent (table 2) as is evidenced by increased acid numbers of the fat in these samples.

The organisms studied were types which are commonly found in cream, and it seems that it is not safe to assume the undesirable types of organisms will be controlled in cream having high titratable acidity. It can readily be seen that any of the organisms studied might cause appreciable damage to the quality of cream even though it was sour. Lactic acid in cream in quantities greatly exceeding the amount normally produced by ordinary milk souring organisms, was definitely not a factor contributing to increased acid numbers in butterfat.

## CONCLUSIONS

1. *O. lactis* and all of the species of bacteria studied were inhibited somewhat by the growth of butter culture organisms in cream; *Myc. lipolytica* showed increased growth in the presence of the butter culture organisms. Lipolysis, even in high acid cream, was extensive enough with all organisms investigated to be of importance in cream quality.

2. *O. lactis*, *Myc. lipolytica* and *Ach. lipolyticum* were definitely inhibited by the addition to cream of excessive amounts of lactic acid. However, they all grew well in cream containing sufficient added lactic acid to give a titratable acidity of approximately 1.0 per cent. The first two species caused lipolysis in cream with an acidity of 2.08 per cent.

3. The addition of lactic acid to sterilized cream in amounts sufficient to increase the titratable acidity up to 2.08 per cent did not cause changes in the acid numbers of the fat after holding 6 days at 21° C.

## RELATION OF VOLATILE ACIDITY OF BUTTERFAT TO RANCIDITY<sup>1</sup>

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In the trials reported in preceding papers the acid numbers of the fat of many samples of fresh cream were determined. Without exception the acid numbers of fresh fat were less than 1.0 and were usually between 0.5 and 0.6. Volatile acidity determinations on these samples of fat invariably yielded such low values that no dependence could be placed on them because they were usually lower than the limit of error of the titration method employed.

### VOLATILE AND NON-VOLATILE ACIDITY RELATIONSHIPS IN THE FAT OF COMMERCIAL BUTTER SHOWING RANCIDITY

Among the many samples of commercial butter examined there were a number which were rancid. Acid number and volatile acidity determinations were made on the fat of these samples.

The data shown in table 1 reveal that the samples of commercial butter which were described as rancid in some degree had acid numbers on the fat ranging from 1.3 to 14.0. Samples 1, 4 and 5 were described as rancid with acid numbers of 4.8, 5.6, and 5.8, respectively, while samples 3 and 9 were described as slightly rancid and had acid numbers of 10.8 and 14.0, respectively. Sample 13 was very rancid and had an acid number of only 1.6 and sample 14 was rancid with an acid number of only 1.3. There was no correlation between the intensity of the rancid flavor and the acid number of the fat.

Samples 1 to 12, inclusive, revealed relatively little variation in the volatile-non-volatile acid relationships of the fat acidity. The percentages of the total acid that were volatile ranged from 11.4 to 16.7. Samples 13 and 14 had very low acid numbers and did not yield a sufficient quantity of volatile acidity to measure accurately by the method employed.

The data show that little or no correlation existed between rancidity and acid number of the butterfat. In the data (table 1), it may be noted that the acid number of the fat and the percentage of the total acid that was volatile was not related directly to the intensity of the rancid odor. While it is rather unsatisfactory to determine the degree of rancidity by organoleptic tests, no other method exists which will detect the defect with equal reliability. Probably the agent responsible for the hydrolysis is an important factor in determining the degree of rancidity that will accompany a certain acid number on the fat. For example, in some experimental trials,

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<sup>1</sup> From a portion of a thesis presented to the Graduate School of Iowa State College in partial fulfillment of the requirements for the Ph.D. degree.

TABLE 1

*Volatile and non-volatile acid relationships in the fat of commercial butter showing rancidity*

Sample	Flavor	Acid number	Volatile acidity*	Per cent of total acid in the fat	
				Volatile	Non-volatile
1	rancid	4.8	0.8	16.7	83.3
2	very rancid	6.2	1.0	16.1	83.9
3	sl. rancid	10.8	1.55	14.3	85.7
4	rancid	5.6	.65	11.6	88.4
5	rancid	5.8	.95	16.4	83.6
6	sl. rancid	2.8	.45	16.1	83.9
7	sl. rancid	3.2	.45	14.1	85.9
8	rancid	2.4	.4	16.7	83.3
9	sl. rancid	14.0	1.9	13.6	86.4
10	rancid	4.8	.55	11.4	88.6
11	rancid	7.6	1.15	15.1	84.9
12	sl. rancid	5.2	.75	14.4	85.6
13	very rancid	1.6	**	..	..
14	rancid	1.3	**	..	..

\* The volatile acidity values represent the milliliters of N/10 sodium hydroxide required to neutralize the acid in 200 ml. of distillate when 10 gm. of fat were steam distilled.

\*\* Quantity too small to measure accurately.

samples of butterfat on which *O. lactis* had acted showed relatively high acid numbers with no indication of rancid odor. It is possible, as has been suggested by Orla-Jensen (6), that certain lipolytic molds are able to consume the volatile acids as rapidly as they are liberated from the fat yielding a fat with a relatively high acid number and yet showing no signs of rancidity.

Hammer (4), Hunziker (5) and others have stated that the odor of rancid butter is due to the presence of some of the lower fatty acids particularly, butyric, caproic and caprylic. Grossfeld and Battay (3) reported that one part of butyric acid in 12,500 parts of a medium could be detected by sense of smell. Stark and Scheib (7) believed that amounts of butyric acid in rancid butter may be so small that though they can be detected in butter by tasting and smelling, they cannot be measured by ordinary chemical means. Since the acid numbers of some of the samples of good butter, as shown in a previous article, were very high and the acid numbers of some of the rancid samples shown in table 1 were low the agencies responsible for rancidity must have exerted a selective action on certain of the glycerides of the fatty acids. Only the higher acids must have accumulated in the good butter showing a high acid number on the fat. Conversely, in the rancid samples having very low acid numbers, a relatively large percentage of the total acid accumulated must have been volatile. From the data presented it is evident that no definite relationship existed between the quantity of acid liberated from the fat and the degree of rancidity present.

The relationship between the volatile and non-volatile acidities of the fat

of samples 1 to 12 of the rancid butter were comparatively uniform regardless of the acid numbers of the fat which ranged from 2.4 to 14.0. There was no relationship between the degree of rancidity and the volatile acidity, that is, in general the slightly rancid samples had the same volatile-non-volatile acid relationship as did the rancid and very rancid samples.

In conclusion, the percentages of the total acid in the fat that were volatile varied only slightly in the samples of rancid butter studied. There was no correlation between the percentages of the total acid in the fat that were volatile and the degree of rancidity.

EFFECT OF THE GROWTH OF VARIOUS LIPOLYTIC MICRO-ORGANISMS ON THE  
PERCENTAGES OF TOTAL ACID OF FAT THAT ARE VOLATILE AND  
NON-VOLATILE

During a study of many cases of rancidity in experimental butter caused by the growth of micro-organisms, an excellent opportunity was afforded to obtain information concerning the relationship between the volatile and non-volatile acidity in the fat of rancid butter. Portions of sterilized sweet cream were inoculated with the organisms to be studied. The cream was then incubated at 13° or 21° C. for 6 days. The acid numbers and the volatile acidities of the fat were determined in the usual manner and the percentages of total acid that were volatile were calculated; the percentages of the total acid that were non-volatile were obtained by difference. The four trials with each organism were purposely not run simultaneously and because of the fact that cultures of different ages were used and different incubation temperatures employed, the degree of fat hydrolysis in the trials was not uniform. The object was to determine whether the organisms would hydrolyze the fat into the same volatile-non-volatile acid relationship under varying conditions of growth.

The average percentage of the acid that was volatile in the four trials with *O. lactis* was 1.9 (table 2). In other trials not reported, slightly higher values were obtained but none of them was over 5.0 per cent. With *Myc. lipolytica* the percentage of total acid that was volatile was higher than with *O. lactis*, the average being 8.4. All the species of bacteria studied gave relatively high volatile acid values and were uniform in their volatile-non-volatile acid relationship. Four trials each with *Ps. fluorescens*, *Ach. lipolyticum*, and *Alc. lipolyticus* gave averages of 14.7, 11.5 and 11.2 per cent, respectively.

There was a relatively close relationship between the volatile and non-volatile acid values in all the trials with an organism, regardless of age of culture used for inoculating the cream, incubation temperature or degree of hydrolysis as shown by the acid numbers. The different organisms varied considerably in the degree of hydrolysis produced, as well as in the percentages of the total acids that were volatile and non-volatile. The *O. lactis* culture, as has previously been shown, was actively lipolytic but the volatile

TABLE 2

*Volatile and non-volatile acidity relationships in butterfat produced by the growth of pure cultures of lipolytic micro-organisms in cream. Sterilized cream inoculated and held 6 days*

Trial	Temperature of incubation	Acid number of fat	Volatile acidity*	Per cent of total acid in the fat	
				Volatile	Non-volatile
<i>O. lactis.</i>					
1	13° C.	18.6	.45	2.4	97.6
2	13°	23.8	.5	2.1	97.9
3	21°	32.8	.6	1.8	98.2
4	21°	26.4	.4	1.5	98.5
Average	.....	.....	.....	1.9	
<i>Myc. lipolytica</i>					
1	13°	9.9	.9	9.1	90.9
2	13°	12.0	1.0	8.3	91.7
3	21°	21.0	1.7	8.1	91.9
4	21°	16.8	1.4	8.3	91.7
Average	.....	.....	.....	8.4	
<i>Ps. fluorescens</i>					
1	13°	3.7	.7	18.9	81.1
2	13°	5.8	.8	13.8	86.2
3	21°	7.6	1.0	13.1	86.9
4	21°	6.5	.85	13.1	86.9
Average	.....	.....	.....	14.7	
<i>Ach. lipolyticum</i>					
1	13°	2.8	.35	12.5	87.5
2	13°	4.7	.5	10.6	89.4
3	21°	7.1	.8	11.5	88.5
4	21°	8.3	.95	11.4	88.6
Average	.....	.....	.....	11.5	
<i>Alc. lipolyticus</i>					
1	13°	5.4	.65	12.0	88.0
2	13°	8.7	1.0	11.5	88.5
3	21°	14.9	1.6	10.7	89.3
4	21°	12.8	1.4	10.9	89.1
Average	.....	.....	.....	11.2	

\* See table 1.

acidity of the fat on which it had acted was somewhat low as compared with all other organisms studied. This observation confirms the belief of Orla-Jensen (6) who suggested that the organism utilized in its metabolism, the volatile fatty acids liberated by its growth.

For reasons previously cited the degree of hydrolysis caused by the same organism in different trials, as determined by acid numbers, varied somewhat. With a few exceptions, all the trials with an organism gave volatile acid values that were quite uniform, regardless of the degree of hydrolysis. In the case of *O. lactis* the acid numbers ranged from 18.6 to 32.8 but the vola-

tile acid percentages varied only from 1.5 to 2.4. With *Myc. lipolytica* the acid numbers ranged from 9.9 to 21.0 and the volatile acid percentages from 8.1 to 9.1. With *Ach. lipolyticum* the acid numbers varied from 2.8 to 8.3 and the volatile acid percentages from 10.6 to 12.5; other bacterial species produced volatile acid values similar to those produced by *Ach. lipolyticum*. The organisms studied varied considerably in the relative percentages of the total acid liberated that were volatile and non-volatile. In all trials with the same organism however this relationship was comparatively uniform.

One organism may have attacked the fat of cream in a somewhat different manner than another, or at least the end products of the metabolic processes were different. With *O. lactis* only a relatively small percentage of the total acid was volatile after the mold had grown while with all bacterial species studied a comparatively large percentage remained after growth. With all the bacterial species studied about the same percentages of the total acid produced from the fat were volatile which in all cases were considerably higher than those produced by either *Myc. lipolytica* or *O. lactis*.

#### ABILITY OF VARIOUS LIPOLYTIC ORGANISMS TO UTILIZE SALTS OF THE LOWER FATTY ACIDS AS THE SOLE SOURCE OF CARBON

Both experimental and commercial rancid butter showed some variation in the volatile-non-volatile acid relationships of the fat. Various workers have suggested that certain micro-organisms utilize some of the lower volatile fatty acids in their growth. In order to determine whether the organisms used in the previously reported experiments could grow in media in which a sodium or calcium salt of a single volatile fatty acid comprised the sole source of carbon, a series of such media were prepared following the general formula of Ayres, *et al.* (1). These media had the following composition:

sodium ammonium phosphate .....	2.0 gm.
potassium chloride .....	.1 gm.
salt of fatty acid .....	5.0 gm.
distilled water .....	1000 ml.

The salts used were sodium and calcium butyrate, calcium caproate and calcium caprylate. Forty ml. portions of each medium were placed in glass containers with screw caps and sterilized in the autoclave. The media were then inoculated with micro-organisms to be studied and incubated at 21° C. After 7 days and also after 14 days of incubation, a complete series of the inoculated media were treated as follows:

The contents of each bottle were placed in a Kjeldahl flask containing 225 ml. of distilled water. Five ml. of N/1 sulphuric acid were added to each flask to free any remaining fatty acid from the salt. The flasks were then placed on the distilling apparatus and heated until 200 ml. of distillate were obtained. These distillates were titrated against N/10 sodium hydrox-

ide, using phenolphthalein as an indicator. Handled in an identical manner, uninoculated 40 ml. portions of each medium served as checks. It was assumed for comparative purposes that any decrease in the volatile acid obtained from a medium after growth of an organism, compared with the check, was due to utilization of the acid by the growing organisms.

There was no apparent uniformity in the ability of different organisms to utilize the fatty acids (table 3). *O. lactis* grew luxuriantly in the media

TABLE 3

*Ability of certain lipolytic micro-organisms to utilize the salts of the lower fatty acids as the sole source of carbon*

Organism	Sodium butyrate		Calcium butyrate		Calcium caproate		Calcium caprylate	
	Days of incubation at 21° C.							
	7	14	7	14	7	14	7	14
<i>O. lactis</i> .....	3.4	1.2	3.7	0.6	6.9	6.8	1.1	0.9
<i>Myc. lipolytica</i> .....	12.1	5.0	11.3	11.0	5.2	4.8	1.3	.3
<i>Ps. fluorescens</i> .....	12.5	12.3	13.0	13.0	4.7	3.6	2.0	1.7
<i>Ach. lipolyticum</i> .....	16.2	16.4	17.0	17.1	7.2	7.2	2.2	1.9
<i>Alc. lipolyticum</i> .....	12.5	9.4	10.2	8.3	7.2	7.2	2.2	1.5
Check (no inoculation) .....	16.1	16.2	17.5	17.1	7.2	7.2	2.9	2.9

The values represent the milliliters of N/10 sodium hydroxide required to neutralize the acid in 40 ml. of medium. The difference between the values given for an organism and the check sample (no inoculation) on the same medium represents the milliliters of N/10 acid utilized by the growing organism.

containing sodium and calcium butyrate and lowered the volatile acid obtainable from the sodium butyrate medium from 16.1 ml. (check) to 3.4 ml. after 7 days and to 1.2 ml. after 14 days. Almost complete disappearance of the butyric acid may be noted. Similar reductions were shown in the calcium butyrate and calcium caprylate media. In the calcium caproate medium some growth was evident but it was not nearly so luxuriant as in the other media. The data further substantiate earlier suggestions that *O. lactis* is able to utilize volatile fatty acids.

*Myc. lipolytica* grew in all the media but grew less luxuriantly than *O. lactis* in the medium containing calcium butyrate, as determined by the titration values after 14 days, the value for *O. lactis* being 0.6 ml. and for *Myc. lipolytica* 11.0 ml. This organism showed greater growth after 14 days in the media containing the calcium salts of caproic and caprylic acids than did *O. lactis*. *Ps. fluorescens* utilized all of the salts to some extent; *Ach. lipolyticum* did not show appreciable growth in any of the media and consequently utilized very little of the fatty acids. *Alc. lipolyticum* utilized sodium and calcium butyrate but was unable to utilize the calcium salts of caproic and caprylic acids to any extent.

The organisms studied varied greatly in ability to use the salts of the lower fatty acids as their sole source of carbon. The fact was established,

however, that some of the organisms studied were definitely able to destroy, by their growth, certain of the volatile fatty acids. Coolhass (2) showed that certain bacteria were able to ferment a large number of fatty acid salts. It is possible also that certain organisms may be able to act on the higher fatty acids in such a manner as to split off acetic acid causing increased titration values. It is therefore evident that the acid number of a fat is not an exact index of the degree of hydrolysis of the fat. Another point to consider is that even though certain organisms utilized the lower fatty acids in synthetic media in which the fatty acids were the only source of carbon, this does not necessarily prove that they would utilize them when growing in cream or butter. Under different circumstances they might obtain their carbon from a more readily available source and leave the fats unhydrolyzed. These results establish the possibility of the utilization of the lower fatty acids by certain micro-organisms growing in cream.

#### SUMMARY AND CONCLUSIONS

1. In samples of commercial unsalted butter showing widely varying degrees of rancidity, the percentages of the total acid in the fat that were volatile varied only slightly; there was no close correlation between the percentages of the total acid in the fat that were volatile and the degree of rancidity.
2. The different organisms studied varied considerably in the percentages of the total fat acid that were volatile and non-volatile. The average percentage of the total acid of the fat that was volatile in the trials with *O. lactis* was 1.9; with *Myc. lipolytica*, 8.4; with *Ps. fluorescens*, 14.7; with *Ach. lipolyticum*, 11.5 and with *Alc. lipolyticus*, 11.2.
3. There was a relatively close relationship between the volatile and non-volatile acid values on the fat in all the trials with each organism, regardless of the age of the culture used for inoculating the cream, the incubation temperature or the degree of fat hydrolysis.
4. Certain lipolytic organisms grew well in media in which a sodium or calcium salt of butyric, caproic or caprylic acid was the sole source of carbon; others grew little or not at all in these media.
5. *O. lactis* grew more luxuriantly in all of the synthetic media than any of the other organisms investigated.

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## HIGH-TEMPERATURE (STEAM-INJECTION) PASTEURIZATION OF CREAM FOR BUTTERMAKING<sup>1</sup>

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Pasteurizers especially designed to remove certain feed flavors from cream have been introduced recently by several manufacturers. All employ direct injection of steam into the cream for heating. By mixing the steam and cream under pressures above atmospheric it is possible to heat the cream to temperatures considerably above the normal boiling point. Rapid cooling is secured by evaporation brought about by drawing the heated cream into a chamber maintained below atmospheric pressure. Constituents of the cream volatile at temperatures below the boiling point of the cream are thus removed at least in part.

Although claims have been made for equipment of this type relative to the destruction of micro-organisms, no published experimental evidence is available. McDowall (1) reported a considerably higher fat content in the buttermilk resulting from churning cream pasteurized in the Vacreator (a steam-injection, high-temperature pasteurizer) than in the buttermilk from cream pasteurized in the ordinary flash pasteurizer.

The work herein reported was undertaken to compare the results secured using one type of high-temperature, steam-injection pasteurizer with those using the standard vat pasteurizer. Factors studied included efficiency in bacterial destruction, effect on fat losses in the buttermilk, and flavor and keeping qualities of the butter.

### DESCRIPTION OF EQUIPMENT

A small, high-temperature, steam-injection pasteurizer built by the C. E. Rogers Company of Detroit, Michigan, of the same design as commercial equipment sold by the same company was made available to the Dairy Division of the University of Minnesota. This equipment was used for the work concerning the fat content of the buttermilk. Due to the ease with which it could be sterilized, the apparatus described by Coulter and Combs (2) was used for the work on bacterial destruction and flavor and keeping quality of the butter. This equipment was designed to operate on the same principle as the Rogers pasteurizer. As shown in figure 1, it consists essentially of a glass tube connected at one end by means of rubber tubing with

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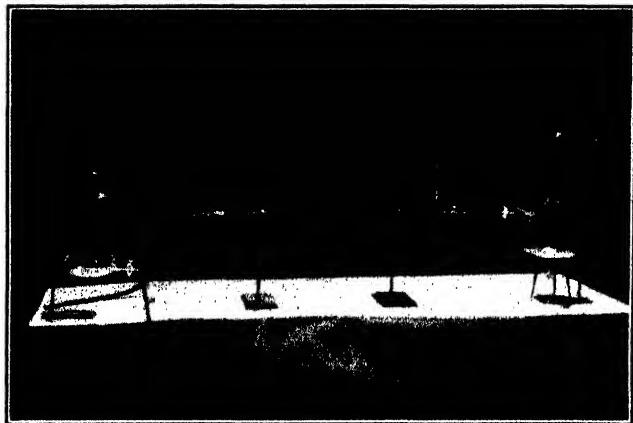


FIG. 1. Laboratory apparatus used for high temperature pasteurization.

sources of cream and steam both under 50 to 60 pounds pressure, and at the other end by the same means to a flask upon which a partial vacuum can be drawn.

The glass tube is 3 feet long and is made of standard 12-mm. O. D. Pyrex tubing. The cream and steam enter the tube through the arms of a Y. Tubes drawn to a 3-mm. orifice are fitted into the arms of the Y in order to cause the cream and steam to meet as sprays. The rate of passage of the heated cream from the tube is regulated by a stopcock placed near the end opposite that at which the steam and cream enter. A thermometer is inserted into the tube by means of a ground glass connection and is held in place by spring clips. Leaving the tube the cream enters a 6-liter Erlenmeyer flask through an opening about half way up the side of the flask. The vapors are drawn off and passed through a surface condenser and a partial vacuum maintained by means of a water aspirator.

In operation steam under pressure is admitted to the tube and after flow is established the outflow is restricted by the stopcock until a temperature of at least 265° F. is reached. Cream under pressure is then admitted to the tube at such a rate as to maintain the temperature at 260° F.

#### EXPERIMENTAL

Lots of both sweet and neutralized sour cream were divided into two parts. One was pasteurized at 260° F. flash by the high-temperature, steam-injection method, and the other by the standard vat process at 160° F. for 30 minutes. Other factors were held as uniform as possible.

*The efficiency of pasteurization.* Many of the bacteria which survive the normal pasteurization process are proteolytic in nature. These organisms are not known to be an important factor in the keeping quality of butter.

However, it is reasonable to believe that insofar as deterioration of butter due to bacterial causes is concerned, a more complete elimination of bacteria than is possible with the methods of pasteurization now in vogue is desirable. The data secured in comparing the efficiency of the vat and high-temperature, steam-injection methods of pasteurization in bacterial destruction are shown in table 1. Plating one ml. of cream direct in the standard plate

TABLE 1

*Efficiency of high-temperature steam-injection and vat pasteurization as indicated by bacterial count using standard plate method*

Sample No.		Pasteurization temperature	Before pasteurization	After pasteurization	Percentage killed
Lot I	I—Sweet cream butter		per ml.	per ml.	
	1	260° F.	253,000	0	100
	2	260° F.	253,000	0	100
	3	260° F.	253,000	0	100
	4	260° F.	253,000	1 on (D)*	100—
	5	260° F.	253,000	1 on (D)*	100—
	6	260° F.	253,000	0	100
	7	160° F., 30 min.	253,000	19	99.99+
Lot II	I—Sweet cream butter		per ml.	per ml.	
	9	260° F.	92,000	0	100
	10	260° F.	92,000	0	100
	11	260° F.	92,000	0	100
	12	260° F.	92,000	0	100
	13	160° F., 30 min.	92,000	8,500	90.8
Lot III	II—Sweet cream butter		per ml.	per ml.	
	1B	260° F.	231,000,000	0	100
	2B	260° F.	231,000,000	0	100
	3B	260° F.	231,000,000	0	100
	4B	260° F.	231,000,000	0	100
	5B	260° F.	231,000,000	0	100
	6B	260° F.	231,000,000	0	100
	7B	160° F., 30 min.	231,000,000	8,500	99.99+
Lot IV	III—Sour cream butter		per ml.	per ml.	
	9B	260° F.	22,400,000	0	100
	10B	260° F.	22,400,000	0	100
	11B	260° F.	22,400,000	0	100
	12B	260° F.	22,400,000	0	100
	13B	160° F., 30 min.	22,400,000	410	99.99+
	14B	160° F., 30 min.	22,400,000	350	99.99+

\* (D)—plated 1 cc. direct.

count, no bacterial growth whatsoever was secured in 18 of the 20 trials with cream pasteurized at 260° F. flash and in the other trials only one colony developed. The results demonstrate a marked superiority in bacterial destruction by this method of pasteurization. Similar results should be secured by pasteurizing cream at the same temperature in commercial equipment; however, cream with a higher bacterial count may be expected due to

contamination following heating from contact with imperfectly sterilized equipment.

*Flavor and keeping quality of the butter.* The cream was churned in sterile, Dazey type, glass churning. The butter granules were washed with sterile water and the butter worked by hand under conditions as nearly aseptic as possible. One-half the churning were salted to contain about 2 per cent salt and the others left unsalted. The butters were scored while fresh and after storage at 40° F. for 2, 8 and 12 weeks. Samples were taken for a standard plate count just prior to each scoring.

The average scores of the butters made from each lot of sweet cream are shown in table 2, and from each lot of neutralized sour cream in table 3.

TABLE 2

*Average scores of butter made from sweet cream pasteurized at 160° F. for 30 minutes and at 260° F. flash*

Lot No.	Pasteurization temperature	Score of butter			
		Fresh	2 weeks	1 month	3 months
<b>I</b>					
Unsalted	260° F.	92	93	93	*
Unsalted	160° F., 30 min.	92	93	93	*
Salted	260° F.	92	93	93	90
Salted	160° F., 30 min.	92	93	93	90
<b>II</b>					
Unsalted	260° F.	92.25	91	92.25	92
Unsalted	160° F., 30 min.	93	*	*	*
Salted	260° F.	93	93	93	90
Salted	160° F., 30 min.	92.5	93	93	90

\* Surface taint—no scores.

TABLE 3

*Average scores of butter made from neutralized sour cream pasteurized at 160° F. for 30 minutes and at 260° F. flash*

Lot No.	Pasteurization temperature	Score of butter			
		Fresh	2 weeks	1 month	3 months
<b>I</b>					
Unsalted	260° F.	90.67	91.5	90.0	89.0
Unsalted	160° F., 30 min.	90.0	90.5	90.0	88.0
Salted	260° F.	91.0	91.18	90.0	88.0
Salted	160° F., 30 min.	90.0	91.0	89.0	87.0
<b>II</b>					
Unsalted	260° F.	90.75	91.0	90.0	88.0
Unsalted	160° F., 30 min.	91.0	91.0	90.0	86.0
Salted	260° F.	90.5	91.0	89.5	88.0
Salted	160° F., 30 min.	90.5	90.0	89.0	86.0

The method of pasteurization did not definitely affect either the initial score or the keeping quality of the butter. The butter made from the cream

pasteurized by the high-temperature, steam-injection method at 260° F. was uniformly criticized as having a slight cooked or custard-like flavor when fresh. This flavor was less apparent in the butter after storage. The butter judges were of the opinion that there was a less marked neutralizer and old cream flavor in the butters made from the neutralized sour cream pasteurized at 260° F. flash. This would be expected if any of the constituents responsible for these flavors are volatile at a temperature below the boiling point of the cream, since the cream boils violently when released into the vacuum chamber of the high-temperature, steam-injection pasteurization equipment. Desirable flavors if volatile would also be partially removed. This factor was not demonstrated to be of importance in this work.

The bacterial counts of the fresh and stored butters are shown in tables 4 to 7. Despite a lower initial count in the unsalted butters made from the

TABLE 4

*Bacterial count by standard plate method of fresh and stored unsalted butter made from sweet cream pasteurized at 260° F. flash, and at 160° F. for 30 minutes*

Churning No.	Pasteurization temperature	Bacterial count of butter			
		Fresh	2 weeks	8 weeks	12 weeks
1	260° F.	0	192	3,900	25,600,000
5	260° F.	1	T.N.T.C.*	T.N.T.C.*	21,600,000
6	260° F.	2	0	2,500	4,600,000
9	260° F.	0	180	1,680	3,500,000
10	260° F.	0	4,200	280	290,000
7	160° F., 30 min.	9	1,560	T.N.T.C.*	46,000,000
13	160° F., 30 min.	350	T.N.T.C.*	680,000	14,800,000

\* T.N.T.C.—Too numerous to count on highest dilution plated.

TABLE 5

*Bacterial count by standard plate method of fresh and stored unsalted butter made from neutralized sour cream pasteurized at 260° F. flash and at 160° F. for 30 minutes*

Churning No.	Pasteurization temperature	Bacterial count of butter			
		Fresh	Stored at 40° F. for		
per ml.	per ml.	per ml.	per ml.	per ml.	per ml.
1B	260° F.	0	2,410	175,000	98,000
2B	260° F.	0	400	89,000	176,000
3B	260° F.	0	80	2	25,000
9B	260° F.	1	30,000	310,000	1,600,000
10B	260° F.	0	20	0	1,000
7B	160° F., 30 min.	1,570	300,000	239,000	1,400,000
13B	160° F., 30 min.	50	50	610	Lost

cream pasteurized at 260° F. flash, the counts after storage were not significantly different than in the unsalted butters made from the vat-pasteurized cream. Bacteria multiply so rapidly in unripened unsalted butter when

TABLE 6

*Bacterial count by standard plate method of fresh and stored salted butter made from sweet cream pasteurized at 260° F. flash and at 160° F. for 30 minutes*

Churning No.	Pasteurization temperature	Bacterial count of butter			
		Fresh	Stored at 40° F. for		
			2 weeks	8 weeks	12 weeks
		per ml.	per ml.	per ml.	per ml.
2 .....	260° F.	1	0	2	200
3 .....	260° F.	1	6	1	100
4 .....	260° F.	0	4	0	100
11 .....	260° F.	0	10	21	0
12 .....	260° F.	11	.10	19	5
8 .....	160° F., 30 min.	6	310	2	1,400
14 .....	160° F., 30 min.	260	27,200	490,000	30,000

stored at 40° F. that a high count after storage is to be expected even with originally very low count butter. Even though cream can be rendered essentially sterile by high-temperature, steam-injection pasteurization, elimination of bacterial deterioration of unripened unsalted butter made from such cream cannot be expected unless contamination after pasteurization can be prevented.

The initial count of the salted butters churned from the cream flash-pasteurized at 260° F. also was lower than that of the butters from the vat-pasteurized cream. The inhibiting effect of salt on bacterial growth was evident as in no case was there an important increase in count during storage. Consequently, the lower initial count of the butters from the cream pasteurized at 260° F. although doubtless desirable, was not in this work a factor in the deterioration of the butter. Although the results might have been different if highly salt tolerant micro-organisms had been present, they are indicative at least of what might be expected under commercial conditions.

TABLE 7

*Bacterial count by standard plate method of fresh and stored salted butter made from neutralized sour cream pasteurized at 260° F. flash and at 160° F. for 30 minutes*

Pasteurization temperature	Bacterial count of butter			
	Fresh	Stored at 40° F. for		
		2 weeks	8 weeks	12 weeks
	per ml.	per ml.	per ml.	per ml.
260° F. ....	1	4	10	2
260° F. ....	1	1	0	0
260° F. ....	0	1	0	0
260° F. ....	0	10	6	4
260° F. ....	0	20	1	2
160° F., 30 min. ....	1,450	290	260	34
160° F., 30 min. ....	62	90	35	23

*Fat losses in buttermilk.* Sweet cream and neutralized sour cream were pasteurized at 260° F. flash, using the small Rogers high-temperature, steam-injection pasteurizer, and other portions of the same cream at 160° F. for 30 minutes by the vat method. After pasteurization the cream in each case was cooled, held over night and churned at 50° F. in a 64-gallon capacity Disbrow churn. The percentage of fat churned which was lost in the buttermilk from the various churning based on the Normal Butyl Alcohol and Mojonnier tests is shown in table 8. In every case the fat lost in the butter-

TABLE 8

*Per cent of fat lost in buttermilk churned from sweet and sour cream pasteurized at 160° F. for 30 minutes and at 260° F. flash*

Lot No.	Pasteurization temperature	Fat test of buttermilk		Per cent* of fat churned lost in buttermilk	
		Butyl Al. test	Mojonnier test	Butyl Al. test	Mojonnier test
Lot I Sour cream	160° F., 30 min.	per cent	per cent	per cent	per cent
	260° F.—I	0.46	0.64	0.93	1.30
	260° F.—II	0.84	0.88	1.95	2.04
Lot II Sour cream	160° F., 30 min.	1.02	1.16	2.37	2.70
	260° F.—I	0.9	0.92	1.65	1.69
	260° F.—II	1.5	1.55	3.14	3.24
Lot III Sour cream	160° F., 30 min.	1.6	1.69	3.35	3.54
	260° F.—I	0.5	0.59	0.91	1.06
	260° F.—II	1.0	0.96	2.29	2.20
Lot IV Sweet cream	160° F., 30 min.	1.1	1.00	2.59	2.29
	260° F.—I	0.6	0.63	0.78	0.82
	260° F.—II	1.7	1.61	2.32	2.19
Lot V Sweet cream	160° F., 30 min.	1.6	1.57	2.18	2.14
	260° F.—I	0.5	0.54	0.94	1.01
	260° F.—II	1.5	1.33	4.57	4.06
		1.6	1.44	4.87	4.39

\* In these calculations the weight of buttermilk was assumed as the weight of cream minus 1.2 times the weight of fat.

milk from the cream vat-pasteurized at 160° F. was much lower than that from the cream flash-pasteurized at 260° F. The difference in actual fat loss was greater than the fat percentages indicate since there was some dilution of the high-temperature, steam-injection pasteurized cream with condensed steam. A part of the water added to the cream in the form of steam is removed by evaporation in the vacuum chamber. Theoretically if the cream is cooled in the vacuum chamber to the same temperature at which it entered the pasteurizer there would be no dilution. Due to heat loss, however, some dilution occurs unless the cream is heated in the vacuum chamber to effect further evaporation. The dilution which occurred in the present work was greater than would result with large commercial equipment and based on the reduction in fat percentage varied from 5 to 15 per cent except for one churning in which the dilution was 26 per cent and another in which

it was 62 per cent. The fat loss percentages shown in table 8 have been corrected to compensate for the dilution. It is evident from the data presented that higher fat losses in the buttermilk may be expected using the high-temperature, steam-injection type of pasteurizer. McDowall (1) has reported similar results with the Vacreator. McDowall attributed the increased fat loss with cream pasteurized in the Vacreator to decrease in the size of the fat globules. Since the steam and cream are mixed under high pressure and since the flow of the heated cream is retarded by a valve in order to build up the pressure, some reduction in the size of the fat globules is to be expected. Whether this is the sole factor involved in the greater loss of fat in churning cream so pasteurized remains to be proven.

There is some evidence that the fat globule "membrane" is altered to some extent by the high heat treatment to which the cream is subjected in the steam-injection, high-temperature pasteurizer. Buttermilk from the cream so pasteurized was observed to be much whiter in appearance than normal buttermilk. Tarassuk (3) found that the brownish tint of normal buttermilk was completely and irreversibly destroyed by heating the buttermilk to a temperature above 180-185° F. Palmer and Tarassuk (4) have pointed out that the brownish tint of natural churned buttermilk is associated with the fat globule "membrane." Tarassuk (3) presented some evidence which indicates that the constituent of the "membrane" responsible for the brownish tint of churned buttermilk is associated with the protein of the fat globule "membrane."

There is no proof nor any intention to suggest that high heat treatment of cream influences its churnability, but if, as appears to be the case, pasteurization at 260° F. in the high-temperature, steam-injection pasteurizer affects the fat globule "membrane," the possibility that the churnability of the cream is influenced thereby merits consideration.

#### CONCLUSIONS

1. Cream which was essentially sterile as determined by the standard plate count was secured from either sweet or neutralized sour cream by pasteurization at 260° F., using a laboratory model high-temperature, steam-injection pasteurizer.
2. Salted and unsalted butter churned from such cream although having a slight cooked flavor while fresh received the same flavor score as butter churned from different lots of the same cream vat-pasteurized at 160° F.
3. Despite lower initial bacterial counts neither salted nor unsalted butter churned from cream pasteurized at 260° F. in the high-temperature, steam-injection pasteurizer kept better in storage at 40° F. than butter from different lots of the same cream vat-pasteurized at 160° F.
4. The fat lost in the buttermilk was much greater in churning cream flash-pasteurized at 260° F. in a small commercial high-temperature, steam-

injection type pasteurizer, than in churning different lots of the same cream vat-pasteurized at 160° F. for 30 minutes.

The authors wish to express thanks to the C. E. Rogers Company of Detroit, Michigan, for the loan of specially designed equipment used in these experiments.

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## OFFICIAL FLAVOR CRITICISMS OF DAIRY PRODUCTS JUDGED IN THE NATIONAL CONTEST

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This analysis of the official flavor judgments of the samples of butter, cheese, milk and ice cream scored in the Students' National Contest in the Judging of Dairy Products is made: 1, that coaches may appreciate more fully the flavors recurring most frequently in the various dairy products; 2, that they may realize the association of or combination of flavors encountered; 3, that they may have an understanding of the relationship between the flavor quality of the product and the number of criticisms made, and 4, that the student may gain encouragement from the fact that although many specific off flavors are possible in dairy products, relatively few are actually encountered in the judging of normal salable dairy products. The period of the study extends from 1927, when ice cream was first introduced into the National Contest, to 1938, inclusive. During these twelve years, seven samples each of butter, cheese, milk and ice cream were judged per year.

Prior to 1930, two or more official judges were selected to place scores and criticisms on each product. Their services were not continued from year to year, although one judge may have served more than one year. Beginning in 1930, with few exceptions, the official criticisms have been placed on the samples by a selected judge for each product, and, beginning in 1932, two "coach" judges have assisted. If the three failed to agree in their judgment of the sample, that sample was not used in the contest. As noted in "History and Development of the Students' National Contest in the Judging of Dairy Products" (1) the official judges have been retained in the same capacity each year, but the same "coach" judges have not been used continuously from year to year. Consequently the flavor criticisms placed on the samples should represent as reliable a composite judgment as it is possible to obtain. However, it must be borne in mind that in many cases specific flavor samples were selected for use in the contest and therefore the percentage distributions reported herein may not apply to commercial products as a whole.

### BUTTER

A study of the official flavor criticisms of butter from 1927 to 1938, inclusive, shows that an average of 1.87 criticisms were made per sample criticized. Sixty-seven, or 79.76 per cent, of the 84 tubs of butter scored were criticized on flavor.

The distribution of the off flavors in butter are presented graphically in

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figure 1. The data show that 22.4 per cent of the flavor criticisms were "old cream"; 15.2 per cent "unclean"; 13.6 per cent "coarse"; 11.2 per cent

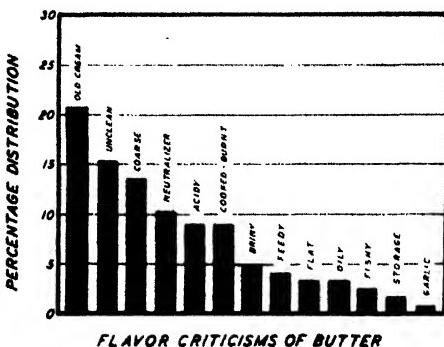


FIG. 1. Distribution of official flavor criticisms of samples of butter judged in The Students' National Contest in the Judging of Dairy Products, 1927 to 1938, inclusive.

"neutralizer"; 8.8 per cent "acidy," and 8.8 per cent "burnt," "cooked" or "heated." These six flavors accounted for 80.0 per cent of all the flavor criticisms. The remaining 20 per cent was divided among "briny," "feed," "flat," "oily," "fishy," "storage," and "garlic" criticisms, with 4.8, 4.0, 3.2, 3.2, 2.4, 1.6 and 0.8 per cent, respectively.

In the above classification some associated flavor criticisms were grouped. For example, "stale" and "cheesy" were grouped with "old cream," "bitter" with "neutralizer," "burnt" with "cooked," and so on.

Inasmuch as an average of 1.87 criticisms per sample criticized was noted, several combinations of flavor criticisms were made. Obviously, if but one criticism were used in the better grade of butter, then at least three criticisms must have been used in the poorer grades in order to maintain such an average. Combinations frequently noted were "coarse," "acidy"; "old cream," "unclean"; "neutralizer," "old cream"; and "neutralizer," "old cream," and "unclean."

The butter was scored by L. S. Edwards and G. A. Gilbert in 1927 and 1928; by L. S. Edwards and H. D. Reynolds in 1929; by H. D. Reynolds and O. A. Storwick in 1930; by H. D. Reynolds and L. D. Reekie in 1931; by C. E. Eckles assisted by coaches E. S. Guthrie and R. E. Roberts in 1932; by C. L. Pier assisted by coaches L. C. Thomsen and F. H. Herzer in 1933; and the remaining five years 1934 to 1938 inclusive by L. S. Edwards assisted each year by two of the following coaches: M. Mortensen, R. E. Roberts, S. L. Tuckey, E. S. Guthrie, L. C. Thomsen, C. M. Mecham, N. E. Fabricius, E. O. Herreid and S. T. Coulter. Thus the official judging was done during the period of this study by six official judges and 11 coach judges (1).

## CHEESE

During the twelve-year period from 1927 to 1938, inclusive, 59.5 per cent of the samples of cheese used in the scoring contests were criticized for flavor by the official judges. A study of the data shows that an average of 1.38 flavor criticisms were made per cheese criticized. The distribution of those flavor criticisms is presented in figure 2. Of the many possible flavor

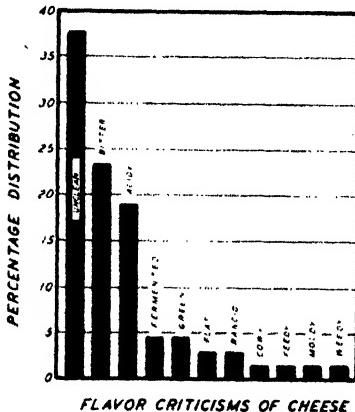


FIG. 2. Distribution of official flavor criticisms of samples of cheese judged in The Students' National Contest in the Judging of Dairy Products, 1927 to 1938, inclusive.

criticisms of cheese three seemed to command the major attention, namely, "unclean," "bitter" and "acydy," which were present 37.7, 23.2 and 18.8 per cent respectively, a total of 79.7 per cent. The remaining 20.3 per cent of the flavors was divided among eight other flavors, of which "fermented," "green," "rancid," and "flat" predominated. Such flavors as "moldy," "cowy," "feedy" and "weedy" were encountered but little in the official samples. When only one flavor criticism was made on the sample, the criticism was generally "acydy," "bitter," or "unclean." If two criticisms were made then "unclean" with either "acydy" or "bitter" were generally used.

The cheese was judged in 1927 by G. N. Tobey and G. A. Gilbert; in 1928 by G. N. Tobey, G. A. Gilbert, and L. H. Marlatt; in 1929 by H. L. Wilson and William White; in 1930 by H. L. Wilson and J. W. Moore; and in 1931 by H. L. Wilson and W. E. Ayres. Since 1931 the cheese has been judged by H. L. Wilson assisted each year by two of the following coaches: W. H. Martin, E. F. Goss, P. A. Downs, G. M. Trout, F. W. Bennett, C. A. Jacobson, R. E. Roberts, S. T. Coulter, H. G. Lindquist, W. H. Sprole, S. L. Tuckey, L. C. Thomsen, and K. R. Renner. A total of seven official judges and thirteen coach judges have placed judgments on the cheese scored from 1927 to 1938, inclusive (1).

## MILK

During the twelve-year period from 1927 to 1938, inclusive, 88.1 per cent of the milk samples used in the national contests were criticized on flavor by the official judges. For each sample criticized on flavor an average of 1.27 criticisms was given. The percentage distribution of the off flavors noted in the samples is shown in figure 3. "Feed" and "cooked" flavors

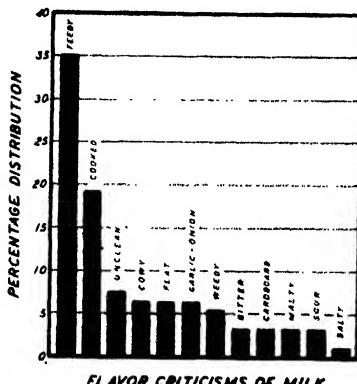


FIG. 3. Distribution of official flavor criticisms of samples of milk judged in The Students' National Contest in the Judging of Dairy Products, 1927 to 1938, inclusive.

dominated the criticisms with 35.11 and 19.14 per cent respectively, a total of 54.25 per cent. Following this group came the "unclean," "flat," "cowy," "onion" or "garlic" and "weedy" flavors, with 7.44, 6.38, 6.38, 6.38 and 5.32 per cent, respectively, a total of 31.90 per cent. The remaining 13.85 per cent was divided among "malty," "cardboard," "bitter," "sour" and "salty" flavors.

Combinations of flavors were not so readily noted in milk as in butter and cheese. When used, however, "feed" was usually one of the flavors given, the combinations being "feed," "salty"; "feed," "unclean"; "feed," "cowy"; and so on.

The milk was officially judged in 1927 by R. J. Posson; in 1928 by C. J. Babcock and R. W. Bell; in 1929 by C. J. Babcock and C. S. Leete; in 1930 by C. J. Babcock and Ernest Kelly; and in 1931 by C. J. Babcock and F. M. Grant. Since 1931 C. J. Babcock has been the official milk judge assisted each year by two of the following coaches: G. M. Trout, L. M. Thurston, L. H. Burgwald, H. G. Lindquist, E. O. Anderson, S. T. Coulter, W. H. Martin, E. L. Fouts, P. H. Tracy, F. J. Doan, T. B. Harrison and I. A. Gould. A total of 18 different judges have placed official judgments on the milk from 1927 to 1938, inclusive (1).

## ICE CREAM

During the twelve-year period from 1927 to 1938, inclusive, 80.95 per

cent of the official ice cream samples in the contests were criticized on flavor. For each sample criticized on flavor an average of 2.04 criticisms were given. The percentage distribution of the flavor criticisms of ice cream during this period are shown in figure 4.

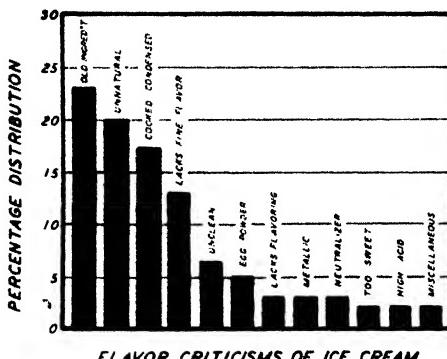


FIG. 4. Distribution of official flavor criticisms of samples of ice cream judged in The Students' National Contest in the Judging of Dairy Products, 1927 to 1938, inclusive.

Four flavors, "old ingredient," "unnatural," "condensed or cooked," and "lacks fine flavor" were noted in 23.02, 20.14, 17.26, and 12.95 per cent of the samples, respectively, a total of 73.37 per cent. "Unclean" and "egg powder" ranked next in percentage incidence with 6.47 and 5.03 per cent, respectively. The remaining 15.13 per cent was about equally divided among "lacks flavoring," "metallic," "neutralizer," "too sweet," "high acid" and "miscellaneous," being 2.87, 2.87, 2.87, 2.15, 2.15 and 2.15 per cent, respectively.

Combinations of flavor criticisms were very frequent, as shown by the fact that when a criticizable flavor was noted an average of two criticisms was given to describe it. Combinations which seemed to recur most frequently were "old ingredient" and "unnatural" or "lacks fine flavor" and "unnatural." Occasionally, another flavor criticism, "unclean," or "cooked," "condensed" or "dry milk," was used also with the combinations of the two previously mentioned.

During the period of the study the ice cream samples were officially judged one year by H. F. Judkins; two years by W. H. E. Reid; one year by P. H. Tracy; two years by A. C. Dahlberg and A. D. Burke; and the remaining seven years by A. C. Dahlberg assisted by two of the following coaches: P. H. Tracy, R. W. Smith, C. A. Iverson, E. L. Fouts, W. H. Martin, J. H. Erb, F. H. Herzer, P. S. Lucas, L. R. Dowd, P. A. Downs, G. M. Trout, and N. E. Fabricius, a total of 17 different judges (1).

#### SUMMARY

A study of the official flavor criticisms of butter, cheese, milk, and ice

cream samples used in the Students' National Contest in the Judging of Dairy Products during the period 1927 to 1938, inclusive, shows that relatively few flavor criticisms are used by the official judges, despite the relatively high percentage of samples criticized in that respect.

Predominating flavor criticisms of *butter* were "old cream," "neutralizer," "unclean," "coarse," "burnt" and "acidy"; of *cheese*, "unclean," "bitter" and "acidy"; of *milk*, "feed," "cooked" and "unclean"; and of *ice cream*, "old ingredient," "unnatural" and "lacks fine flavor." With the possible exception of milk, two or more criticisms were used to describe the flavor of the lower scoring samples.

The result of this study and analysis of trends in official flavor judgments is in no way intended as a guide to future scoring, but merely to classify and make available the flavor criticisms for those who may not have access to the official scoring records. Inasmuch as the samples used were selected in many cases for a specific flavor, the percentages distribution reported herein may not necessarily apply to commercial products as a whole. However, the flavor criticisms encountered in these studies appear to be representative of those encountered in the commercial products throughout the country.

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# THE USE OF ANNATTO AS A TRACER IN CREAM FOR MANUFACTURING PURPOSES AND ITS DETECTION\*

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Many municipalities permit the importation and sale of two grades of cream, namely, cream for fluid consumption which has been separated from milk produced under the requirements for the production of fluid milk, and cream for manufacturing purposes, produced under less stringent requirements. Cream for manufacturing purposes usually contains 40 per cent butterfat and is often shipped from distant points. On most markets cream for manufacturing purposes can be purchased cheaper than cream produced for fluid sales. For economic reasons, some milk dealers may be tempted to obtain and use cream intended for manufacturing purposes for a part or all of their fluid sales. The use of manufacturing cream for fluid purposes not only constitutes a violation of a particular ordinance, but results in unfair competition between dealers and increases the surplus over fluid sales, thus reducing prices paid producers.

The legal addition at the point of shipment of a denaturing agent or tracer to cream for manufacturing purposes would discourage the use of such cream for fluid purposes. Doan (3) reports that the State of Pennsylvania requires the addition of sugar or salt to cream and milk from uninspected sources going into the manufacture of ice cream and butter, respectively.

Alkaline annatto (vegetable cheese color)<sup>1</sup> is suggested as a universal tracer for addition to milk or cream intended for manufacturing purposes. The addition of 2 ml. of annatto to 10 gallons of cream is recommended. Two ml. in 10 gallons is equivalent to 1 part in 18,927. This concentration of annatto does not cause any perceptible change in color nor any change in flavor of the cream. A slight difference in color can be noted only if exceedingly careful comparisons are made. Annatto is easy to add and its presence in cream can be readily detected.

It is recommended that the annatto be added to the cream while in the pasteurizer. This method of addition permits thorough mixing and avoids the possibility of spilling annatto on the cans. Annatto may be added directly to the cream in cans. When added in this manner, considerable care

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<sup>1</sup> Hereinafter referred to as annatto.

must be exercised to get exactly 2 ml. in each 10 gallons and to thoroughly agitate each can after the addition of the annatto, to insure proper mixing. Care should also be exercised to prevent spilling annatto on the cans, thus causing an untidy appearance.

#### DETECTION OF ANNATTO

A method for the detection of annatto in milk and cream is given in the A. O. A. C., 4th Edition (4). This method has proven satisfactory on milk and on low testing cream. However, experimental work done at this station has shown this method is not satisfactory when applied to 40 per cent cream because of the high ratio of fat to casein. It was therefore necessary to devise a method for the detection of annatto in 40 per cent cream.

#### METHOD

Transfer 10 ml. of ethyl alcohol to a glass-stoppered flask, and add 10 ml. of the prepared cream sample. Shake vigorously for 20 seconds. Add 20 ml. of aviation grade gasoline<sup>2</sup> (without lead). Shake vigorously for 20 seconds. Add 0.5 ml. of formaldehyde and shake a few seconds. Transfer to a centrifuge tube and centrifuge in an unheated machine for 30 minutes at the speed (or faster) recommended for the Babcock test. By means of a pipette withdraw the alcohol-serum layer and transfer it to a small beaker. A piece of rubber tubing attached to the pipette will permit the operator to hold the tube before the eyes and thus observe when all the serum has been drawn into the pipette. Fold a 4 cm. Whatman No. 3 filter paper and place in a small (1 inch) funnel. Saturate the paper with NaOH immediately before use by placing 1 ml. of 5 per cent NaOH in the filter allowing it to run through. Filter the solution through the prepared paper. Do not allow the filter to become empty until filtration is complete, otherwise filtration will be slowed up by increased viscosity and by gumming of the filter. Open the paper and wash with a gentle stream of water. Dry the filter with or without heat. Treat paper with a few drops of a 10 per cent aqueous solution of  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  (containing sufficient concentrated HCl to make the solution acid and a small amount of metallic tin to keep the solution reduced). Dry the paper without heat and examine by transmitted light. The appearance of any pink coloration confirms the presence of annatto. The test requires two hours to complete and is sensitive to 1 part in 500,000.

#### EXPERIMENTAL AND DISCUSSION

The cream used in this work contained 37 to 43 per cent fat. Ten samples of annatto as supplied by 5 manufacturers were used. Since alkaline annatto dyes the casein in the cream, the essential problem was to extract the annatto and obtain it in concentrated solution. It was also necessary

<sup>2</sup> 73 Octane was used in this work.

simultaneously to separate the fat to aid in breaking the emulsion, and to promote alcohol-serum separation.

*Extraction of fat and annatto.* On standing, or after centrifuging, the mixture will consist of three separate layers. The gasoline merely extracts the fat from solution and with the fat rises to the top of the mixture. The middle layer consists mostly of casein. The lower layer contains the alcohol, serum, and annatto if present. Obviously, annatto must not be soluble in the fat solvent, and fat must not be soluble in the annatto solvent. Fat solvents experimented with included carbon disulphide, benzene, xylene, tetra chloroethane, carbon tetrachloride, chloroform, acetone and petroleum ether. Annatto solvents experimented with included ethyl, methyl, and tertiary butyl alcohols. The solubility of annatto in a number of other alcohols was nil or slight.

*Formaldehyde.* The use of formaldehyde is not necessary. It does, however, harden the casein layer sufficiently to prevent it from disintegrating when inserting the pipette and thus permits easier removal of the alcohol-serum layer.

*Speed of centrifuging.* If time is not a factor, centrifuging is not necessary. Definite separation will occur if the mixture is permitted to stand 8 hours. When separation is accomplished by gravity, the use of a separatory funnel is recommended to permit drawing off the serum-alcohol layer. When testing cream, centrifuging 30 minutes at Babcock speed is sufficient. Higher speeds can be used satisfactorily and are necessary when testing highly stabilized ice cream mixes.

*Filtration.* During filtration the fibers of the filter paper absorb the annatto. The absorptive powers of different papers vary. Twenty-four grades of paper were used. Of these, Whatman No. 3 gave the best results. A 4 cm. paper is used to concentrate the annatto within a relatively small area. When milk serum is made alkaline, its viscosity increases and it becomes slightly adhesive. If the filter is permitted to become empty during filtration the sodium hydroxide in the paper tends to form an adhesive substance with the serum and to clog the paper. For most rapid filtration it is recommended that the filter be not permitted to become empty. After washing any gummy material from the filter, the paper will be dyed a straw yellow, providing annatto is present in a concentration greater than 1 part in 150,000. In greater dilutions there will be no apparent change in the color of the paper.

*Drying the filter.* After washing, the filter paper may be permitted to dry or if desired may be dried by means of a hot plate. However, care must be taken to prevent scorching or charring. After treatment with stannous chloride a pink coloration may be observed immediately providing the concentration of annatto was greater than 1 part in 250,000. When annatto is present in lower concentrations, it is necessary to dry the paper without heat and to examine it for traces of color by means of transmitted light. The

light source consisted of an ordinary 60 watt lamp placed 4 inches from a 3 cm. hole cut in cardboard. The color, when present, becomes more apparent after 8 to 12 hours.

*Standardization of annatto.* Attempts to determine a minimum standard strength of annatto for use as a tracer in cream by comparisons with a standard color solution have not proven successful. Various annatto solutions were diluted in tenth normal sodium hydroxide to the same concentration, and were compared in a Bausch and Lomb colorimeter. Some of the diluted solutions appeared identical, yet when the undiluted solutions were added to cream to make a concentration of 1 part in 500,000, it was not possible to detect the presence of certain brands of annatto. Dilute annatto solutions which appeared identical on examination in the colorimeter were then examined in a photometer. When two matched dilute solutions prepared from the same annatto solution were compared, the photometer indicated they were identical. When any two matched dilute solutions prepared from different annatto solutions were compared, the photometer indicated they were nearly identical in some instances and widely different in other instances. These results indicate that the various annatto solutions used contained at least two different dyestuffs present in different proportions. These different proportions, however, resulted in the same apparent color as determined in the colorimeter. It has also been shown by Barnicoat (1) by Carrie (2) that commercial annatto is a mixture of two or more dyestuffs and that the mixtures are not always in the same relative proportions. The substance producing positive results in the test outlined is probably the potassium salt of norbixin.

It is recommended that standardization of annatto for use as a tracer in cream be based on its detection in cream by the method outlined, the annatto to be of such strength that its presence can be detected in 40 per cent cream when diluted with 40 per cent cream to 1 part in 500,000. One of the 10 samples of annatto used could not be detected in cream when diluted with cream to 1 part in 500,000.

When annatto is added to cream in the ratio of 2 ml. to each 10 gallons, then 10 gallons of such cream would have to be mixed with more than 260 gallons of cream not containing annatto in order to produce a cream in which the annatto present could not be detected. The possibility of detecting annatto in cream in this extreme dilution would deter most "would-be" unethical milk dealers from using manufacturing cream for fluid sales.

*Quantitative determination of annatto.* The qualitative test for annatto can be made roughly quantitative by a comparison of the color obtained on the filter paper with colors obtained from known concentrations. When annatto is present in concentrations greater than 1 part in 100,000 the product should be diluted with cream to produce a less brilliant color, and the dilution factor used in the calculation of concentration. Dilution to the limit of sensitivity can also be applied in determining concentration.

*Effect of various factors on the test.* The effect of length of time of storage, freezing, acid production, neutralization, heat, and homogenization on the sensitivity of the test were studied.

Samples of cream containing various concentrations of annatto were treated as follows:

- (a) Stored for a period of 2 years at 0° and 29° F., respectively.
- (b) Permitted to sour until the per cent acid ranged from 0.10 to 0.80, and pH ranged from 6.6 to 4.3.
- (c) Samples ranging in acidity from 0.11 to 0.80 per cent were neutralized to 0.10 per cent acid, using sodium sesquicarbonate.
- (d) Heated to 190° F. and held for 10 minutes.
- (e) Homogenized at 145° F. up to 4,000 lbs. pressure per square inch.

The annatto was as readily detected in all samples treated as indicated as it was in freshly prepared samples containing the same concentrations of annatto.

#### SUMMARY

Annatto (vegetable cheese color) is recommended as a universal tracer for use in cream intended for manufacturing purposes. A test for the detection of annatto in cream has been devised. This test is based upon the simultaneous extraction of fat with gasoline and extraction of annatto with alcohol, removal of alcohol-serum by centrifuging, absorption of annatto by an alkaline filter, and confirmation with stannous chloride. The sensitivity of the test is not affected by length of time of storage, freezing, acid production, neutralization, heat, or homogenization. The test is sensitive to 1 part annatto in 500,000 parts of cream.

#### ACKNOWLEDGMENT

The authors wish to acknowledge the cooperation of the manufacturers of annatto who generously supplied the annatto used in this work.

#### ADDENDA

Regulations of the Health Department of the District of Columbia as approved by the Commissioners of the District of Columbia March 7, 1939, require the addition of 4 ml. of annatto to each 10 gallons of milk, cream or ice cream mix shipped into the District of Columbia to holders of Manufacturer's Permits. Proposals for changes are now before the commissioners to reduce the amount of annatto to 2 ml. for 40 per cent cream, 1 ml. for 20 per cent cream, milk and ice cream mix.

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## MILK ENERGY YIELD AND THE CORRELATION BETWEEN FAT PERCENTAGE AND MILK YIELD

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There is a considerable literature on the correlation between fat percentage and milk yield and its meaning. Krizenecky (1) compiled the published results up to 1934 for various breeds of cattle in America and Europe. He gives the following summary for 58 sets of data:

	Minimum	Maximum	Average
Variability in milk yield, $V_M$ .....	11.00	31.73	21.67
Variability in fat percentage, $V_f$ .....	3.83	12.69	8.58
Coefficient of correlation, $r_{Mf}$ .....	-.016	-.506	-.199

The values under minimum do not necessarily, or probably, represent a single set of records; likewise under maximum. Krizenecky seems to feel that the average figures may be taken as typical of the results as found in practice for the two variables, fat percentage and milk yield.

The purpose of the present paper is to develop an estimate of the expected value of  $r_{Mf}$  on the basis of the theory that milk yield is inversely proportional to milk energy per unit milk, or milk-energy yield is independent of milk composition. It is necessary to use some approximations in this development. The purpose is not to develop a means of computing  $r$  but to develop a means of testing whether or not the constant energy theory holds good in data where the available figures are limited to the above statistical constants (plus mean fat percentage,  $\bar{f}$ ). This, again, is not to be taken as a substitute for direct determination of the relation between fat percentage and milk-energy yield where the necessary observations are available. It is a matter of connecting fat percentage with milk-energy yield where only the statistical constants,  $V_f$ ,  $V_M$ ,  $\bar{f}$  and  $r_{Mf}$  are given. To use Krizenecky's average figures we shall have to assume a value for  $\bar{f}$  but this does not detract too seriously in the outcome.

### MILK-ENERGY YIELD AND $r_{Mf}$

Milk energy per unit of milk may be estimated<sup>1</sup> as directly proportional

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<sup>1</sup> The estimate of milk-energy yield is commonly made in terms of 4 per cent milk (FCM) by the formula  $FCM = M(.4 + .15f)$  or some modification of that formula (2, page 405). Bearing on the accuracy of the estimate may be cited 107 observations (4-week periods, Holstein cows) by the Pennsylvania Institute of Animal Nutrition in which milk-energy yield was directly determined by calorimetry. The coefficient of correlation between the determined caloric yield and FCM yield computed by the above formula from the directly determined milk and fat yields works out to be .997. The range in actual

to  $2\frac{2}{3} + f$ . If we have a set of records of milk yield and fat percentage for a population of cows in which the trend of milk-energy yield is horizontal when plotted against fat percentage,  $r_{Mf} = 0$ , the regression of milk yield on fat percentage is given by the equation,

$$M = \frac{K}{2\frac{2}{3} + f} \quad (1)$$

in which  $K$  is a constant representing the average milk-energy yield of the particular population. Equation (1) when plotted gives a curved line as illustrated diagrammatically in figure 1. The slope of the line varies throughout its course according to the equation,

$$\frac{dM}{df} = -\frac{K}{(2\frac{2}{3} + f)^2} \quad (2)$$

To the same set of records let us fit a straight line by the method of correlation. This straight line will cut the curved line of equation (1) somewhat as shown in figure 1, and its slope,  $S$ , will be,

$$S = r_{Mf} \cdot \frac{\sigma_M}{\sigma_f} \quad (3)$$

The symbol,  $S$ , is used in place of  $\frac{dM}{df}$  in equation (3) to avoid possible confusion with the  $\frac{dM}{df}$  of equation (2).

The slope of the two lines will be equal at or near the mean fat percentage. Let us say the slopes are equal at the mean fat percentage, then, from equations (2) and (3),

$$-r_{Mf} = \frac{\sigma_f}{\sigma_M} \cdot \frac{K}{(2\frac{2}{3} + \bar{f})^2} \quad (4)$$

In place of  $\frac{\sigma_f}{\sigma_M}$  we may write  $\frac{V_f \bar{f}}{V_M \bar{M}}$ , in which  $\bar{M}$  is the mean milk yield of the particular population. As a close approximation we may say  $M = \frac{K}{2\frac{2}{3} + \bar{f}}$ , neglecting the small difference that exists as shown in figure 1. Substituting in equation (4) and rearranging we have

$$-r_{Mf} = \frac{V_f \bar{f}}{V_M} \frac{2\frac{2}{3} + \bar{f}}{K} \frac{K}{(2\frac{2}{3} + \bar{f})^2}$$

Note that  $K$  cancels out. The relation is independent of the absolute value of  $K$ , or average level of milk-energy yield in the particular population. We have then the equation,

$$-r_{Mf} = \frac{V_f}{V_M} \frac{\bar{f}}{2\frac{2}{3} + \bar{f}} \quad (5)$$

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milk-energy yield per cow per day is from 1700 to 19850 calories. Holstein cows may yield as high as 38000 calories per day by 4-week periods. If the range used covered 0 to 38000 calories the coefficient of correlation would undoubtedly build itself up still closer to unity.

as an approximate expression of the correlation between fat percentage and milk yield in any set of records in which milk-energy yield is independent of fat percentage,  $r_{FCM_f} = 0$ . Conversely, if in a certain population the observed correlation between fat percentage and milk yield is substantially that given by equation (5), the constant energy theory holds good for that population.<sup>2</sup>

This converse phase is of special interest because the published results of investigations on the correlation between fat percentage and milk yield often do not include a direct computation of the correlation between fat percentage and milk-energy yield, or the original data in sufficient detail for a direct computation; but the statistical constants involved in equation (5) may be included. If application of equation (5) to these constants gives a larger negative value to  $r_{M_f}$  than that found directly from the observations, it indicates a positive sign for  $r_{FCM_f}$ ; while a smaller negative value indicates a negative sign for  $r_{FCM_f}$ . If the difference between the direct  $r_{M_f}$  and the  $r_{M_f}$  by equation (5) is small it is proof that  $r_{FCM_f}$  is of small magnitude.

#### MILK-PROTEIN YIELD AND $r_{M_f}$

The relation between milk-protein yield and milk-energy yield is so nearly one of direct proportionality (3) that it is probable milk-protein yield could be substituted for milk-energy yield in the above development. Perhaps milk-protein yield is representative of the mammary machinery operating in the process of lactation, while milk-energy yield is representative of the work done by the lactating cow. The estimate of milk-protein yield from known milk and milk-fat yields is subject to greater error than is the similar estimate of milk-energy yield, and this, in the absence of direct protein determinations, favors the use of milk energy rather than milk protein.

#### DISCUSSION

Returning to Krizenecky's summary of 58 sets of records with respect to fat percentage and milk yield and the correlation between them, we find a range of  $r_{M_f}$  from  $-.016$  to  $-.506$ . At one extreme is a set of records in which fat percentage and milk yield are independent ( $r_{M_f} = -.016$ ); at the other extreme a set in which (perhaps) fat percentage and milk-fat yield are independent.<sup>3</sup>

<sup>2</sup> According to equation (5) we expect to find a larger negative value for  $r_{M_f}$  in a population of high-testing cows (as Jerseys) than in a population of low-testing cows (as Holsteins). Observed facts agree with this expectation.

By a development similar to that of equation (5) we find  $r_{F_f} = \frac{V_f - 2\bar{f}}{V_f(2\bar{f} + \bar{f})}$ . The expected (on the constant energy theory) correlation between fat percentage and milk-fat yield is positive and smaller numerically than that between fat percentage and milk yield, where  $V_f = V_M$  and  $\bar{f} > 2\bar{f}$ . Observed facts agree with this expectation.

<sup>3</sup> If fat percentage and milk-fat yield are independent,  $r_{F_f} = 0$ , we find by approximations similar to those of equation (5),  $-r_{M_f} = V_f/V_M$ . Krizenecky does not give  $V_f$  and

The average of the correlations is  $r_{Mf} = -.20$ . Applying equation (5) to the average figures (assuming  $\bar{f} = 4$ ) we find an expected correlation of  $r_{Mf} = -.24$ . Using the average figures the observed and expected correlations are in good enough agreement ( $-.20$  vs.  $-.24$ ) to say that, typically, the constant energy theory holds good. The range found in  $r_{Mf}$  indicates the typical relation, is subject to a good deal of disturbance in the various sets of records as observed. These disturbances are presumably of an artificial nature, operating in either direction at random.

Stated (somewhat theoretically) another way, the populations of  $r_{Mf}$ ,  $r_{Ff}$ , and  $r_{FCMf}$  each fall into a normal distribution curve;  $r_{Mf}$  with its mode at  $-.24$ ;  $r_{Ff}$ , at  $+.16$ ;  $r_{FCMf}$ , at zero.

As to the low value of  $r_{Mf}$ ,  $-.2$ , equation (5) suggests that this is associated with the low ratio of variability in fat percentage to variability in milk yield. If we could eliminate the variability in milk yield due to factors other than composition of the milk and deal with a population of cows, embracing a wide range of fat percentage we might find  $V_f = V_M$  and by equation (5) if  $\bar{f} = 4$ , expect<sup>4</sup> to find  $r_{Mf} = -.6$ .

*The fact that the correlation between fat percentage and milk yield is low does not detract from its biological significance or warrant its neglect in a practical breeding philosophy.<sup>5</sup>* For biological purposes (nutrition, ge-

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$V_M$  definitely for the particular set of records in which  $r_{Mf} = -.506$  and we can only say (approximately) that  $r_{Ff} = 0$  in this set of records if  $V_f/V_M = .506$ .

\* In an actual case (2, page 423) dealing with average records for several years (instead of annual records) of individual cows we find  $V_f/V_M = .6$  (instead of  $.4$  as usual in annual records) and  $r_{Mf} = -.44$  (expected,  $r_{Mf} = -.36$ ). The point is that, dealing with records, as between individual cows, on the basis of the average of several years has reduced variability in milk yield and has not changed variability in fat percentage appreciably, as compared with single-year records. As expected (on the constant-energy theory) we find a relatively large observed value for  $r_{Mf}$ .

There is a limit at which equation (5) breaks down. For example, if  $V_f/V_M = 2$  and  $\bar{f} = 4$  equation (5) gives  $r_{Mf} = -1.2$ . Evidently the approximations fail us too badly at some point. However, experience with numerous actual sets of records in which  $r_{Mf}$ ,  $r_{Ff}$ , and  $r_{FCMf}$  are directly computed shows that equation (5) gives sensible results. Indeed, it serves as a rough check on the accuracy of the arithmetic of the computations.

<sup>5</sup> An adequate discussion of the genetic and other biological implications of the correlations cannot be attempted here. The commonly low observed value of  $r_{Mf}$  has given rise to the breeding philosophy that fat percentage and milk yield are independent. If such a philosophy were sound it would be very essential to breed cows giving milk of high fat percentage since, other things being equal, a 6-per-cent cow would yield as much milk as a 3-per-cent cow and twice as much milk fat! Only in a strain giving milk of high fat percentage could we hope, under such a philosophy, to attain maximum yield of milk solids or milk energy. It is true, of course, that as between various cows the yields of milk and various milk constituents (milk, water, protein, fat, lactose, ash, solids, calories) are all highly correlated one with another. If we accomplish an increase in the yield of any one we almost inevitably accomplish an increase in the yield of each of the others.

A particularly elusive feature of the relation between fat percentage and milk yield appears in dealing with the regression of fat percentage on milk yield. If  $r_{Mf} = -.2$ ,

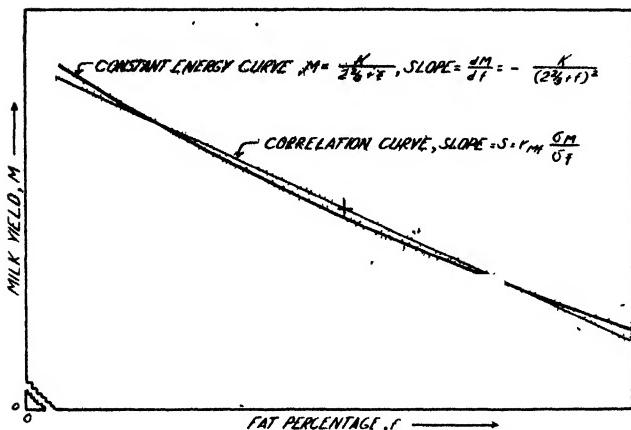


FIG. 1. Diagram in explanation of the derivation of equation (5).

The abscissas represent fat percentage; the ordinates, milk yield. The stippling represents the area of a population, but it is not intended to represent density of population.

The curved regression line has the equation,  $M = \frac{K}{2f+1}$ . It is fitted to the individual

records of the population by least squares,  $K = \bar{FCM}/15$  or  $K = (20/3)\bar{FCM}$ , the symbol,  $\bar{FCM}$ , indicating the mean of the individual FCM yields of the population.  $M$  and  $FCM$  are reckoned in the same unit of weight. All points on this curve have the same milk-energy value and hence it is referred to as a constant energy curve. The theory that this curve represents the typical regression of milk yield on fat percentage, as between comparable individual cow records, is referred to as the constant energy theory. Milk energy yield and FCM yield are used interchangeably, as justified in footnote 1.

The straight regression line has the equation,  $M = a + \left(r_{mf}\frac{\sigma_m}{\sigma_f}\right)f$ . It is fitted to the individual fat percentage milk yield points of the population by the method of correlation or least squares. The curve of this equation, so fitted, must go through the means of fat percentage and milk yield, marked in the diagram by a cross. It frequently happens in actual cases that the linear regression cuts  $M=0$  below  $f=10$ . Obviously in such cases the linear regression will not bear extrapolation to  $f=10$ . This suggests that regression within the population limits of  $f$  may be curvilinear, after the order of the constant energy curve.

The constant energy curve, cuts the mean milk yield at the weighted mean fat percentage,  $\Sigma mf/\Sigma M$ . The correlation or straight line regression cuts the mean milk yield at the unweighted fat percentage,  $\Sigma f/n$  or  $\bar{f}$  as symbolized. There is a small difference between the two averages and accordingly the constant energy curve does not go through the cross in the diagram.

In developing equation (5) it is assumed the two regression lines have the same slope at the unweighted mean fat percentage. It is also assumed that milk yield by the equation of the constant-energy curve at the unweighted mean fat percentage is the same as the actual mean milk yield of the particular population, neglecting the existing small difference, which amounts to less than .5 per cent of the mean milk yield. If these approximations are accepted equation (5) follows rigorously. The converse follows, if somewhat less rigorously at least with great assurance in natural data: if the correlation between fat percentage and milk yield in a particular population of records conforms to equation (5) the constant energy theory holds good for that population, that is  $r_{mf}=0$ .

netics, etc.) we need a measure of milk yield which is independent of milk composition. Such a measure is afforded by milk-energy yield. It is not afforded by milk yield itself. It is not afforded by milk-fat yield.

#### SUMMARY

The theory that milk yield, so far as affected by fat percentage (or milk composition), is inversely proportional to milk energy per unit of milk (or milk-energy yield is independent of milk composition) is used to develop the expected coefficient of correlation between fat percentage and milk yield,

$$r_{Mf} = \frac{V_f}{V_M} \frac{\bar{f}}{2\% + \bar{f}},$$

where  $V$  is variability

and  $\bar{f}$  is mean fat percentage. For  $V_f/V_M = .4$  (the usual value as  $r_{Mf}$  has been generally derived) and  $\bar{f} = 4$  the formula gives  $r_{Mf} = -.24$ . The low value of  $r_{Mf}$  found in practice is in keeping with the constant energy theory, and does not detract from its biological significance or warrant its neglect in a practical breeding philosophy.

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$\sigma_f = .3$  and  $\sigma_M = 3000$  pounds the regression coefficient is  $-.00002$ , that is, an increase of 5000 pounds in milk yield is accompanied by a decrease of only .1 in fat percentage. This seems to be practically negligible.

In the biological use of the convenient term "fat percentage" it should always be held clearly in mind that fat percentage is a number expressing the average rate of milk-fat secretion, as a part, relative to the average rate of milk secretion as a whole.

## BLOAT IN DAIRY CATTLE\*

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The danger from bloat has been an important deterrent in pasturing legumes, especially alfalfa, red clover, and sweet clover. These legumes have proven excellent for grazing, where they can be established without too great an initial cost. The carrying capacity, palatability and effect on the flavor of milk of legumes have been favorable to their use. However, the danger of losing animals from bloat has kept many farmers from pasturing legumes more generally than is practiced at present.

Despite the fact that bloat, hoven or tympanitis has been recognized for a long time, very little experimental evidence is available as to the cause or of infallible preventive measures. A large number of preventive measures has been reported in the literature. These measures may prove successful in some communities under certain conditions, while not effective under similar conditions in other communities. Even on adjoining farms the same preventive measures may not be equally effective. Bloat may be prevalent some years and show no evidence in others, for instance for several years no bloat was experienced with cows on experimental legume pastures at South Dakota State College. The cows were fed grain, and not allowed to go on the pastures when hungry. This practice proved effective as a preventive measure against bloat for several years but in later years the cows bloated on both alfalfa and sweet clover pastures although the management of the cows had not been changed. The same pasture plots were used and the cows were fed grain before turning on the pastures as in previous years.

### REVIEW OF LITERATURE

Bloat occurs more frequently on alfalfa and red clover than on sweet clover and according to some was not the same when diagnosed (1). Others have found that bloat may be caused by any kind of feed, such as spoiled silage, roots, etc. (2). When red clover is cut and fed as a soiling crop it reduces or eliminates the danger from bloat which attends its use as a pasture (3). The bloating of cattle from alfalfa in Argentina is not considered a very serious menace when rock salt is available at all times (4). Sheep are less likely to bloat on alfalfa which is mature than on young succulent growth (5). Data from 431 farms which had pastured sweet clover for an average of nine years, with 9884 cattle, 15,721 head of sheep, reported bloat

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occurred on 35 farms or nine per cent. The losses were no greater than might be expected from other causes. The losses were greater in irrigated and the more humid sections. Few cases of bloat were reported from pasturing the first year's growth of sweet clover (6). A survey indicated that bloat on sweet clover was worse when the young growth from the second year sweet clover is soft and succulent (7). Welch, March and Tunnicliff (8) report the results of a survey on the occurrence of bloat in sheep and cattle pastured on sweet clover. This report indicated that a thick luxuriant stand is more likely to produce cases of bloat than a scattered more stemmy growth. They found that cattlemen may expect a loss of less than 1 per cent on sweet clover pasture, and sheepmen may expect a loss of about 0.5 per cent. No particular system of managing the stock on the pasture seemed to have any influence on bloat occurrence.

Viljoens (9) defines acute tympanitis or hoven, "as an abnormal accumulation of gases in the large stomach producing great distention of the organ with subsequent paralysis of its walls." The causes are: 1, Accumulation of gases naturally formed as the result of obstruction in the gullet; 2, excessive formation and accumulation of gases in the stomach owing to causes originating in the stomach itself—(a) food stuffs easily fermented, (b) too rapid and too large consumption of green food, (c) wet, wilted, lucerne or other green food. Veech (10) states that bloat or hoven in cows is due to succulent foods eaten under certain conditions which cause the formation of large quantities of gas in the rumen or paunch, and in consequence the swelling of the left flank. It is most often seen when cattle are turned hungry on to such succulent green feed as lucerne, clover, trefoil, etc., or when cattle gorge themselves on wet grasses or herbage. He found that some cattle seem to be more subject to bloat than others. Healy and Nutter (11) attribute bloating to the sugar in the blossoms of plants. Their analysis showed that red clover blossoms contained 3.6 per cent sugar, alfalfa blossoms 2.8 per cent, and white clover blossoms 2.4 per cent, whereas the leaves of clover and alfalfa contained less than 1 per cent of sugar. When the clover blossom were ground and mixed with distilled water and held at a temperature of 37° C. for 24 hours they found an active fermentation took place. At the end of this period 45 per cent of the volume of the original clover-blossom mass of carbon dioxide, had formed. Ohman (12) found that bloat was brought about by excessive ingestion of easily fermenting foods, *e.g.*, clover, lucerne, peas, and quick growing cereal crops. Dixon (13, 14) states that, "acute bloating of ruminants, cattle particularly, may occur at any time from a variety of causes but most commonly through turning hungry cattle on to luxuriant green feed, or on to herbage country, after heavy rains and when the young herbage is making rapid growth." The editor of the "Queensland Journal" (15), in discussing bloat, attributes the cause to the filling of the paunch with gas, which causes paralyses of the muscles of the paunch, and inhibits peristalsis, which aids in belching or

expulsion of the gases from the stomach. He states, "It is possible that the cyanoglucoside content of the plant is a contributing factor to gas formation." "Hoard's Dairyman" (16), in an editorial, asserts that bloat appears more frequently in May and June and late fall, and that young animals are more subject than old animals, cattle and sheep more susceptible than horses, and hungry animals more susceptible than well-fed animals. Legumes wet with dew or rain, or frosted or wilted are likely to produce bloat. Plants grown on poor soil and low in minerals, notably lime, may be a factor in causing bloat. The editorial asserts further that, "The embryonic cells of the rapidly growing shoots of legumes contain easily fermented material, which is an important factor in bloat. Cells in the leaves are largely embryonic in nature and can be quickly broken down. If legumes are covered with hoar frost the cell walls are completely permeable. If eaten at that time, the enzyme balance is certain to be disturbed and the digestive action will start before the cell can reestablish its enzyme balance. The respiratory rate of this type of cell is very rapid and respiration is accompanied by the liberation of large quantities of carbonic acid gas." Kephart (17) after making a wide survey summed up the situation of bloat as follows:

1. Bloat is worse during May and June when the young growth from the second year sweet clover is soft and succulent.
2. Bloating is more apt to occur between 4 and 7 P.M.
3. Bloating is most apt to occur when animals are turned on pasture when hungry. Apparently they eat too greedily and the stomachs cannot take care of the large amount of material when it starts to ferment.
4. Young cattle suffer more than old ones, probably because they don't know when to stop eating.
5. Bloating seems more likely to occur when animals do not have ready access to water, or do not have an adequate quantity of minerals.
6. Cattle are principal sufferers from bloat.
7. Bloating seems to occur more frequently in some districts than others. This may be due to the lack of minerals in the soil.
8. Bloating is to some extent an individual matter with animals.
9. Bloating seems to occur more frequently in wet years than in dry years, and when plants are covered with rain or dew.

Schalk (18) used two fields of sweet clover and two fields of alfalfa. One field of each legume was equipped with a watering system with which the grass was well watered; the other was maintained under natural conditions of moisture. Comparable groups of animals were used on each type of pasture.

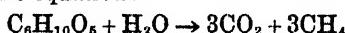
Before the animals were turned on the pasture, they were subjected to a number of different environmental factors such as:

1. Complete fill with dry feed in lot.
2. Complete fill with ordinary pasture (mostly bluegrass).

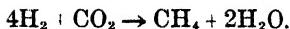
3. Partial fill under conditions of 1 and 2.
4. Fasted in dry lot for 12 to 18 hours and with and without water.
5. Fasted in dry lot for 12 to 18 hours and heavily salted.
6. Placed on pasture at different times of day, with heavy dew and following a rainfall.

No perceptible excessive intra-ruminal fermentation was observed in the animals placed on either pastures, following any of these preparatory environmental factors. Bloating does not appear to occur very frequently if cows are left on the pasture continually, so they never get very hungry. Other feeds in the pasture seem to reduce the danger of bloat.

Hutyra, March and Maminger (19) state, "The composition of gases in the rumen differ according to the character of the fodder and the consequent nature of the fermentations. There is always a large amount of carbonic acid gas (50 to 80 per cent of the total gas) then sulphuretted hydrogen, nitrogen and oxygen, the two last derived from the swallowed air, although oxygen is also formed during fermentation." Tappeiner (20) analyzed the gases from the rumen of an ox, two goats and a suckling lamb, and reported 45 to 67 per cent  $\text{CO}_2$ ; 31-34 per cent  $\text{CH}_4$ ; 0.19-7.1 per cent  $\text{O}_2$ ; 0.19-4.7 per cent  $\text{H}_2$ ; 1.9-15.2 per cent of  $\text{N}_2$ . Langwitz (21) analyzed ox-rumen gas and found 4 per cent  $\text{O}_2$ ; 2-19 per cent  $\text{N}_2$ ; 40-50 per cent  $\text{CO}_2$  for cabbage leaf feeding, and 70-80 per cent for alfalfa, clover and grass; 16 per cent  $\text{CH}_4$  for buckwheat and 34 per cent  $\text{CH}_4$  after vetch feeding. Symons and Buswell (22) conducted methane fermentation on 45 substances, including carbohydrates, alcohols, acids, aldehydes, ketones, etc. It was concluded that the fermentation is an anaerobic oxidation reduction involving water and catalyzed by bacteria. The primary products are carbon dioxide and hydrogen, the two combining to form methane and water. Woodman (23), working in artificial media seeded with cultures of bacteria, has demonstrated the existence of two distinct anaerobic cellulose-fermenting organisms. The first breaks down cellulose to hydrogen and carbon dioxide, together with organic acids. The products of decomposition by the second type of organisms are methane, carbon dioxide and organic acids. Popoff (24) demonstrated in 1875 that methane formation in ruminants was attributable to micro-organisms since ruminants digest cellulose in the absence of cytase. Barker (25) suggested that methane is always formed through a reduction of carbon dioxide. Boruff and Buswell (26) studied the production of carbon dioxide and methane from anaerobic fermentation of corn stalks and suggested the equation:

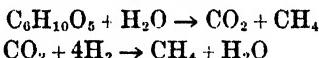


The fermentation reaction was one of reduction and oxidation involving the addition of  $\text{H}_2\text{O}$  and forming carbon dioxide and hydrogen. Methane was formed by the combination of hydrogen with some of the carbon dioxide according to the equation:



Fisher, Tronz, Lieske, Rudolfe (27) state that mixtures of CO and H are transformed into CH<sub>4</sub> in the absence of air by the action of certain bacteria (Gram positive non-spore forming bacteria).

Barker (28) in his quantitative study of methane fermentation, showed that the source of methane is always the reduction of carbon dioxide and that carbon dioxide results from the oxidation of alcohols. Christensen (29) found that alcohols, methane, hydrogen and carbon dioxide and other products are produced by the fermentation of cellulosic materials. Buswell, Boruff, Claire (30) state that methane can be produced by subjecting cellulose materials, such as corn stalks, cooked with a reagent such as lime-water to render it readily susceptible to bacterial action and then subjected to an anaerobic fermentation with bacteria such as are found in the sludge of sewage. Tappeiner (31) reported the production of 4.7 grams methane per 100 grams of cellulose digested. Kellner (32) reported 3.17 grams methane per 100 grams of starch digested, 5.45 grams CH<sub>4</sub> per 100 grams straw pulp, 429 grams CH<sub>4</sub> per 100 grams of mixed ration. Washburn and Brody (33) state, "It is generally known that considerable anaerobic carbohydrate fermentation occurs in the rumen with methane and carbon dioxide as the principal end products." The intermediary reactions whereby these end products are formed are not clearly known.



Woodman and Stewart (34) assert that cellulose fermenting bacteria possess a dual mechanism whereby they first hydrolyze cellulose to glucose and then ferment the glucose to organic acids and gases. The gas-producing activities of the micro-organisms may be circumscribed by the resulting acidity of the rumen contents. Ritzman and Benedict (35) carried out experiments to determine the methane production of the horse, pig and goat. The pig produces no methane because he subsists largely on concentrates and tubers. The goat when fed on roughage produces about the same as the cow, and the horse produces about half as much.

Dougherty (36) approached the problem of bloat from the angle of type of gas produced, rather than the quantity of gas production and the subsequent intraruminal pressure. He found that 50 mm. to 90 mm. above atmospheric pressure increased blood pressure, the heart beat, and caused dyspnea, but was not the direct cause of death. Intra-ruminal pressure did, however, increase the rate of absorption of carbon monoxide and carbon dioxide from the rumen. The rumen practically ceased all movements and eructation was inhibited. These symptoms were in evidence with very low concentrations of carbon monoxide in the rumen. Hydrogen sulphide was also found in traces in the rumen gas, by Dougherty. The hydrogen sulphide appeared in greater concentrations in the liquid portion of the ingesta.

Injections of small amounts of hydrogen sulphide into the rumen of sheep proved fatal without additional pressure. Experimental evidence according to Dougherty indicates that "the concentration and the rapidity of absorption of the two toxic gases, carbon monoxide and hydrogen sulphide, from the stomach of ruminants has an important bearing on the symptoms of bloat, and in some cases may be the cause of death."

McCandlish (37), in a survey among dairy farmers in Scotland, discusses the factors which induce bloat, methods of prevention and treatment. The discussion is similar to material already reviewed. He makes the assertion that "bloat appears to be most common on the heavier and better soils of the district." He also states that "Good soils at fair elevation will generally be found to be more closely associated with bloat, than similar soils at a lower level." These conclusions are based on observations rather than experimental data.

#### EXPERIMENTAL

For a number of years gas from bloated cows has been collected with the thought of determining the kinds of gases and the percentage of each. It

TABLE 1  
*Summary of gas analyses*

No. of samples	CO <sub>2</sub>	Ill.*	O <sub>2</sub>	H <sub>2</sub>	CO	C <sub>2</sub> H <sub>6</sub>	CH <sub>4</sub>	N <sub>2</sub>
<i>A. Gas Analysis from Cows Bloated on Sweet Clover</i>								
9	61.97	0.12	3.60	0.31	0.44	0.09	15.30	18.20
<i>B. Generated Gas from Sweet Clover Plants</i>								
6	60.74	0.04	2.79	9.36	0.17	0.00	0.14	26.18
<i>C. Gas Analysis from Cows Bloated on Alfalfa</i>								
1	59.80	0.00	3.61	0.05	0.05	0.00	18.42	18.07
<i>D. Generated Gas from Alfalfa Plants</i>								
12	53.49	0.05	1.53	26.51	0.20	0.05	0.05	18.11
<i>E. Generated Gas from Sudan Grass Plants</i>								
7	52.65	0.22	2.54	24.81	0.13	0.00	0.00	19.65
<i>F. Generated Gas from Sorghum Plants</i>								
5	58.64	0.14	0.80	34.58	0.08	0.00	0.08	5.68
<i>G. Generated Gas from Corn Plants</i>								
2	18.30	0.15	9.83	7.16	0.18	0.00	0.00	64.38
<i>H. Generated Gas from Marsh or Lowland Grass</i>								
1	25.20	0.35	0.93	14.35	0.58	0.00	0.00	58.59
<i>I. Generated Gas from Brome Grass</i>								
1	24.66	0.00	3.64	4.24	0.50	0.00	0.00	66.96

\* Ill.—Illuminants.

was hoped that by ascertaining the kind of gases produced, some clue to the cause of bloat might be found. Samples of gas were collected from bloated cows by means of a long rubber tube, which was inserted into the gullet and

thence to the rumen of the cow. Rubber gas bags and gas collecting tubes were used to collect and hold the gas until it was analyzed. The cows fought the insertion of the rubber stomach tube, and therefore some of the gas samples collected by this method were more or less contaminated with air. At other times the rumen was so filled and the pressure so great that particles of the macerated sweet clover or alfalfa were forced into the tube, necessitating withdrawing the tube to force the materials out.

Six samples of gas were secured from cows which had died from bloat. These samples were taken with a trocar and the gas collected in rubber gas bags. The gas secured in this manner was less contaminated with air than the samples of gas secured by means of the rubber tube inserted into the gullet.

A number of samples of gas were secured from legumes and non-legume plants by fermentation in the laboratory. No difficulty with bloat was experienced with non-legumes; however, it was thought advisable to ascertain the gas from these plants for comparison with the gas from plants which were known to cause bloat. The plants were collected at different stages of maturity, as well as at different times of the day, to note if that had any effect on the amount or kind of gas produced. The materials were cut, and ground with a pestle and mortar, then put in a flask and 1000 cc. of distilled water added. The generating apparatus was placed in an incubator with the temperature maintained at 37° C. When the gas container, which held a solution of 80 cc. of 3 per cent H<sub>2</sub>SO<sub>4</sub>, and 20 grams of Na<sub>2</sub>SO<sub>4</sub>, was filled with gas the glass stoppers were firmly closed and the gas stored until analyzed. The rate of generation of gas of the various plants was noted.

The Orsat apparatus for the gas analysis was used and the method of procedure outlined in Technical Bulletin 320, Department of Commerce, Bureau of Mines, was followed. A general view of the apparatus is shown on page 5 of the bulletin, therefore no attempt will be made to describe the apparatus used in the analyses. The method of procedure is also discussed completely in the bulletin, and therefore will not be outlined herein.

#### DISCUSSION

One sample of gas was collected from a cow which had been grazing on bluegrass pasture. The cow's horns became fastened in a woven wire fence while she was being watered, which held her muzzle under the water. She undoubtedly was forced to take in considerable water before being released, and died immediately. The gas was taken direct from the rumen, immediately after the cow died. The analysis indicated 68.12 per cent CO<sub>2</sub>, 17.20 per cent CH<sub>4</sub>, 12.73 per cent N<sub>2</sub>, 1.80 per cent O<sub>2</sub>, 0.05 per cent CO, 0.10 per cent illuminants. The analysis of the gas resulting from the conditions described above is similar to the gas analyzed from cows which bloated on sweet clover and alfalfa, yet the cow had been pasturing on a bluegrass pasture. The cause of death was not bloat, but according to the

attending veterinarian, the ingestion of large amounts of water, and finally the filling of the lungs with water.

The analysis of the gas from animals which were bloated, as well as those which had died from bloat, indicated a relatively high percentage of carbon dioxide and methane. There was no appreciable difference in the analysis of the gas samples taken with the stomach-tube and those taken by means of the trocar through the rumen wall. The difference obtained was probably due to the greater contamination from the air when the stomach-tube was used.

According to the reported analyses in tables 1A and 1C, there seems to be no significant difference in the analysis of the gas when cows bloat on sweet clover or alfalfa pastures. The number of samples of gas analyzed from alfalfa pasture is so limited that a definite conclusion on this point cannot be drawn. According to Farmers Bulletin 1653 bloat from sweet clover when diagnosed has turned out to be something entirely different than bloat from alfalfa, red clover or alsike. If this is true the difference does not appear to be in the kind of gas produced.

The analysis of gases from sweet clover and alfalfa plants, generated under laboratory conditions, shown in tables 1B and 1D, are quite similar. These plants were gathered at different times of the day as well as at different times during the pasture season. When the analysis of the gas obtained from bloated cows is compared with the analysis of gas secured from plants generated under laboratory conditions it is observed that the percentage of methane in the latter is practically negligible whereas the gas from bloated cows contains an appreciable percentage of methane. The analysis of gas from non-leguminous plants, which rarely produce bloat in cows under normal conditions, indicate no methane, except in the case of sorghum shown in table 1G.

The presence of methane in the rumen of cattle when not bloated was demonstrated as early as 1875 (24). Other workers (20, 21, 25, 31, 32, 33) have demonstrated that methane is present in the rumen of cattle and that methane results from the fermentation of cellulose materials under anaerobic conditions (22, 23, 26, 27, 28, 29, 30, 31, 33, 34).

Inasmuch as both carbon dioxide and methane are produced under normal feeding conditions in the rumen of cattle, these gases in themselves are not the direct cause of death from bloat. The carbon dioxide and methane may be responsible for the excessive pressure sustained in the rumen and as a consequence the cessation of eructation, and the normal movements of the rumen. When the gases cannot be eructed they are absorbed. The absorption of carbon dioxide and the small percentage of carbon monoxide in the rumen may prevent the hemoglobin of the blood from carrying oxygen. It is known that when carbon monoxide comes in contact with the blood by diffusion, it unites with the red pigment of the blood corpuscles, to form a relatively stable compound.

The analyses shown in tables 1B, 1D, 1E, 1F, 1G, 1H and 1I from both legumes and non-legumes under laboratory conditions show approximately the same percentage of carbon monoxide. Carbon monoxide is toxic to animals (38). It would seem, therefore, that non-legumes should produce bloat as well as legumes. The fact that cattle rarely bloat on non-legumes under normal conditions, may be due to the lower intra-ruminal pressure when non-legumes are eaten.

Dr. Dougherty in a personal letter to the writer states that in recent experimental work he has found traces of hydrogen sulphide, and this gas together with carbon monoxide in the rumen has an important influence on the symptoms of bloat, and in some cases may be the cause of death.

According to McNally (38), hydrogen sulphide acts on all animals through all tissues, especially the lungs. It produces labored breathing, pains in the stomach and death by coma. Concentrations of hydrogen sulphide above 0.1 per cent inspired air terminates in respiratory failure. In concentrations over 0.2 per cent breathing is paralyzed and asphyxia ensues.

Lange's "Handbook of Chemistry" states that "hydrogen sulphide in 0.1-0.2 per cent by volume kills most animals in a very short time."

In the gas analyses heretofore reported, no effort was made to test for hydrogen sulphide in either the gas of bloated animals or in the gas generated under specified laboratory conditions. Hydrogen sulphide results from the destruction of vegetable and animal matter; therefore, is probably present in the gases normally excreted by animals. In the case of bloat it is possible that the hydrogen sulphide is absorbed directly from the rumen similarly to the other gases, when the intra-ruminal pressure reaches a point where rumen activity is inhibited. In view of the fact (38) that low concentrations of hydrogen sulphide are fatal to animals, the absorption of it directly from the rumen may be a significant factor in the death of animals from bloat.

#### SUMMARY AND CONCLUSIONS

1. Analyses have been completed on gases from cows which have bloated from sweet clover and alfalfa. These gases were collected by inserting a stomach-tube into the gullet of the cows. The gases were collected in gas rubber bags, and in glass, and metal gas tubes. A number of samples of gas were collected from cows which had died from sweet clover and alfalfa bloat. These samples were collected directly from the rumen through the trocar cannula.

2. Samples of gas were collected from legume and non-legume plants generated under laboratory conditions. These samples were analyzed for the same gases as the samples from bloated animals.

3. No significant differences were indicated in the analyses of the gases from sweet clover and alfalfa plants.

4. No significant differences were discernible in the analyses of the gases

from legume and non-legume plants generated under laboratory conditions, except in the per cent of carbon dioxide in the corn plant, lowland and brome grasses. The small number of samples analyzed may account for this difference.

5. The significant difference in the analyses of the gases from bloated animals and those generated under laboratory conditions both from legume and non-legume plants, is in the methane content of the gas from bloated animals. The methane in the gas from bloated animals averaged about 17 per cent, whereas most of the samples of the gas generated in the laboratory showed no methane, and those which did indicated a negligible percentage.

6. Carbon monoxide, a toxic gas, was present in all the samples analyzed. No significant differences obtained in the per cent of carbon monoxide in the gas from different sources.

7. The percentage of gases indicated by the analyses do not in themselves point to the cause of bloat, or the cause of death from bloat. It is possible that the rapid formation and absorption of gases when legumes are eaten is due to a series of factors none of which will cause bloat or death in themselves. That is, it is known that carbon monoxide and hydrogen sulphide are highly toxic gases. These gases are produced under normal feeding conditions, in non-legumes as well as legumes yet are not fatal to the animal. The other gases present in the rumen gas are negative in their effect on the animal, yet these gases may be responsible for inducing the conditions which make the toxic gases fatal by increasing the intra-ruminal pressure or inhibiting the normal functioning of the rumen.

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## ABSORPTIVE CAPACITY OF DIFFERENT MATERIALS ORDINARILY USED FOR BEDDING

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Bedding is used to keep cows clean, comfortable, and to absorb the liquid excrement. It is important, therefore, for the dairy farmer to know which materials meet these requirements at the lowest cost.

In the grain-producing sections of the United States, the cereal straws provide most of the bedding used, with shredded or cut corn stover added in the corn belt region. Shavings and sawdust are used in sections of the country where these are available at reasonable prices and where the cereal straws are high-priced and scarce. In many of the European countries such materials as peat, peat moss, and dried leaves are used. The latter products are seldom used in this country for bedding because of their scarcity and relatively higher price. The hulls of the cereals and buckwheat as well as the straws of buckwheat and flax are used as bedding in areas where these plants are grown on a commercial scale.

There is probably little difference in the "comfort effect" of the several materials used for bedding. If sufficient bedding is used any of the materials given will provide adequate comfort and protect the animals from cement surfaces or other materials which might be used for floors in stalls or stanchions.

The important consideration in beddings, therefore, is their absorptive capacity. Beddings which absorb the liquid excrement are an important factor in keeping the cows clean as well as conserving the urine, which constitutes an important part of the manurial value of the total excrement from livestock.

### DISCUSSION

According to the Pennsylvania Experiment Station, the urine contains about half the nitrogen and three-fourths of the potash of the fertility elements of the manure.

Michigan circular 25 gives the following amounts of excrements voided daily by cows and the fertilizing elements contained therein:

*Amount of excrement voided per day of 24 hours (1)*

Solid excrements	Liquid excrements	Total excrements
lbs. 49	lbs. 19	lbs. 68

*Fertilizing elements in the excrement*

	Nitrogen	Phosphoric acid	Potash
	per cent	per cent	per cent
Solid excrement .....	0.43	0.12	0.04
Urine .....	1.05	trace	0.36

*The daily amount and composition of solid and liquid excrement (2) voided by mature animals*

	Pounds per animal		Nitrogen		Phosphorus		Potassium	
	Solid   Liquid		Solid   Liquid		Solid   Liquid		Solid   Liquid	
			per cent		per cent		per cent	
Cattle	52.0		19.4	3.24   0.95	0.090   0.012	0.124   0.79		

Work from the South Dakota Station (3) indicated the following amounts of solid and liquid excrements voided by lactating cows.

*Average daily amount of solid and liquid excrement  
(Average of 4 cows for 20 days)*

Solid	Liquid
grams daily 17,823.30 (approx. 39.3 lbs.)	grams daily 9072.2 (approx. 20.1 lbs.)

The above cows were receiving a balanced ration of alfalfa hay and medium ground oats.

These data together with those given above, indicate the importance of conserving the liquid excrement if the farmer is to realize the manurial value from the animals maintained.

It is apparent that the manurial value of the excrement varies in amount and in its chemical constituents depending on the kinds of feed, the age and kind of animal fed. Young growing animals take more nutrients out of the feed to build bones and tissues than mature animals. A lactating cow uses considerable of the elements in the feed to produce milk. A mature animal which is not producing, or using nutrients in production, voids in the excrement a smaller percentage of the chemical elements in the feeds consumed than animals which are immature and producing.

Regardless of the varying value of the liquid excrement from cows, its value is important enough in maintaining the fertility of the farm to conserve it as well as is practically possible.

**ABSORPTIVE POWER OF BEDDING MATERIALS**

In five trials at the South Dakota Station (3) ten pounds whole and chopped oat, wheat and rye straws, pine shavings and sawdust were placed in a beet-pulp sack. The sacks were placed in a barrel containing sufficient

urine to completely submerge the material. Weights were placed on the sacks to assure complete covering by the urine. The sack with the material was allowed to soak for about two hours, and then suspended and allowed to drain until dripping ceased (usually from two to three hours). The materials in each sack was then emptied into a tub and weighed.

The same procedure was followed in which the bedding materials were submerged in well-water to note differences, if any, in the absorptive capacities of the two liquids. The following tables indicates the results:

*Urine absorbed by ten pounds of bedding\**

Oat straw		Rye straw		Wheat straw		Shavings	Sawdust
Whole	Cut	Whole	Cut	Whole	Cut		
lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
17.65	19.62	16.43	15.88	17.20	13.66	12.10	23.28

\* Average of six trials.

*Water absorbed by ten pounds of bedding\**

Oat straw		Rye straw		Wheat straw		Shavings	Sawdust
Whole	Cut	Whole	Cut	Whole	Cut		
lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
18.73	19.37	17.0	17.23	18.73	18.30	11.16	22.40

\* Average of three trials.

These data would seem to indicate there is little if any difference in the absorptive capacity of the cereal straws. The slight differences which are indicated by the data may be due to other factors, such as the moisture content of the straws used. Although the straws used appeared to be dry, it is possible that there might have been a slight difference in the moisture content. Moisture determinations were not made on the straws. The various trials were carried on over a period of several weeks. During this time the building in which the straws were soaked and allowed to drain was heated by a furnace and the humidity of the air might have varied enough to account for the differences in weight.

There seems to be no appreciable absorptive difference in whole and cut cereal straws. Inasmuch as straws are usually cut in order to make them go further, this would seem to be an erroneous idea. Inasmuch as the absorptive capacity is the important criterion on the amount of bedding to use, the cut straw would not go any further than whole straw so far as the amount of urine absorbed in concerned.

Another fallacy is that shavings will absorb more liquid than straws. These data do not indicate so. The shavings were mixed shavings and were dry, having been stored in the loft of the hay barn for several years.

The sawdust was principally from soft-wood, and drier than most sawdust used for bedding. It was secured from the local carpenter shop and had not been exposed to moisture.

The data showed no appreciable difference between the amount of urine and well-water absorbed by the cut and whole cereal straws, sawdust and shavings.

The work at Maryland (4) indicates a somewhat higher absorptive capacity than the data from the South Dakota Station excepting for sawdust which the author explained was due to the fact that the sawdust was very high in moisture. The trials were, however, conducted in a different manner, which may account for a part of the difference.

*Water absorbed per pound of bedding of different materials (4)*

Cut stover	Wheat straw		Shavings	Sawdust
	Whole	Cut		
lbs.	lbs.	lbs.	lbs.	lbs.
<i>On basis of ten pounds, comparable to work at S.D. station</i>				
25	20.0	20.0	22	8.0

Whisenhand (5) reports a trial in which five to seven pounds of different bedding materials were soaked in water for twelve hours, suspended in a room in the barn until dripping had ceased, and weighed after five and twenty-four hours. The following table indicates the results of the trial:

Materials	Water by 100 pounds of the material after 24 hours	Relative hold- ing power after 24 hours
Whole oat straw .....	lbs.	
Whole oat straw .....	240	100
Cut oat straw .....	244	97.6
Wheat straw .....	210	84.0
Mixed shavings, Chicago carload .....	119	47.6
Mixed shavings, from local planing mill .....	130	52.0
Mixed sawdust, from local planing mill .....	160	64.0
Fine dry, white pine shavings .....	185	74.0

These data indicate a somewhat higher absorptive capacity for wheat and oat straws both whole and cut. The mixed shavings data check very well with the South Dakota results. These trials were conducted similarly to the South Dakota Experiment Station trials except for length of draining and soaking time.

Herbert (6) reports the following absorptive capacities of litter:

*Water retained by 100 kg. of material after 24 hours*

Kinds of material	kg.
Wheat straw .....	220
Barley straw .....	285
Oat straw .....	228
Sawdust of poplar wood .....	435

These data also indicate a higher retentive capacity of the cereal straws than the data from the South Dakota Station. As previously stated, the moisture content of the straws used by the South Dakota Station was not determined. The straw was secured from stacks and was dry. The data quoted from other experimenters did not indicate whether the moisture content of the straws was known or not. It is assumed that air dried straws were used and no attempt was made to measure the moisture in the straws nor the moisture which might have been absorbed from the air.

No explanation can be offered for the lower absorptive capacities of the cereal straws used in the South Dakota trials. The time allowed (approximately two hours) for the straws to soak would seem sufficient to permit of thorough soaking, particularly when the trials were conducted in a building of approximately 60° F. temperature, and the bedding materials completely submerged. Because of the relatively lower rainfall in this territory the air-dried straw would at least be as free from moisture as straws used in the trials quoted.

The absorptive capacity of the shavings as reported in these trials checked very closely with the results from other stations.

**SUMMARY**

Ten pounds of oat, wheat and rye straws cut and whole were soaked from one to two hours in sufficient urine to completely submerge the sacks containing the straws. The same procedure was followed in which well-water was used instead of urine. The materials were allowed to soak for one to two hours and then suspended and allowed to drain until dripping had ceased, after which the content of the sack was emptied into a tub and weighed.

The increase in weight of the bedding materials was assumed to be due to the amounts of urine or water retained by the various materials.

No appreciable difference in absorptive capacity was noted between cut and whole straws, and straws from different cereals. Such minor differences as are indicated might be due to the completeness of soaking, or draining. The temperature and humidity of the room in which the materials were soaked and allowed to drain were not maintained at the same point, which might result in a difference in weight. That the original moisture content of the materials used for bedding affect the absorptive capacity of the materials is indicated by the sawdust used in the Maryland experiments.

The data from the South Dakota Station indicate that the whole cereal straws provide satisfactory litter. Because of the abundance and cheapness of these straws, they can be recommended in preference to any other materials for this area. The manurial value of the cereal straws is also greater than the manurial value of sawdust or shavings, materials ordinarily substituted for cereal straws.

The data did not indicate greater absorptive powers for cut straws; therefore, it would not seem advisable to cut cereal straws for bedding except where storage room for straw is at a premium.

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## American Dairy Science Association Announcements

### THIRTY-FIFTH ANNUAL MEETING, PURDUE UNIV., W. LAFAYETTE, IND., JUNE 24-28

#### HOUSING ACCOMMODATIONS

Adequate, commodious and convenient housing facilities will be available in the Cary Residence Halls and the Union Club of the Student Union Building. The Residence Halls were just completed in November, 1939, and are beautifully and pleasantly furnished with fine comfortable lounges and living quarters. The rates in the Residence Halls will be \$1.25 per person per night with everything furnished. The rates for children under 12 years of age will be 75¢ per night. The rates in the Union Club which has regular hotel facilities will be from \$2.50 and up per person per night. Rooms will also be available at the hotels in Lafayette.

Reservations should be made in writing to K. C. Boxell, Dairy Department, Purdue University, West Lafayette, Indiana.

#### REGISTRATION HEADQUARTERS

Registration Headquarters will be maintained in the lobby of the Purdue Memorial Union Building, beginning June 24, 1940.

#### LAST CALL FOR PAPERS

Titles and Abstracts of Papers to be presented at the June meeting must be in the hands of the Program Committee not later than April 15. Send all titles and abstracts to Dr. B. E. Horrall, Department of Dairy Husbandry, Purdue University, W. Lafayette, Indiana.



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## THE CAROTENE CONTENT OF SEVERAL HERBAGES DURING THE GROWING SEASON\*

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During the past few years an increasing tonnage of various forage crops is being preserved by the ensiling process. Very few studies, however, have been made to determine at what stage of growth these crops contain the greatest amount of carotene. If this information were available it might be possible to ensile certain crops when the carotene content is high. A high carotene roughage is especially desirable in feeding dairy cattle in order to produce a milk of high vitamin A content.

Virtanen and co-workers (10) reported that the total quantity of carotene of a plant increased rapidly up to the time of blooming and then diminished as the plant matured. In a later report Virtanen (9) indicated that carotene attained a maximum value just before or at the beginning of flowering, but that it may be increased by proper and adequate fertilization or it may be decreased by the same factors that retard growth. Hauge (4) found that young alfalfa, 10 to 12 inches high, contained almost twice as much vitamin A as did the alfalfa in full bloom. Hilton, Hauge and Wilbur (5) stated that young alfalfa, 10 to 12 inches high, contained 90 units of vitamin A per gram but in the bloom stage it contained only 70 units. They also noted that soybeans, 12 to 15 inches high, contained 54 units of vitamin A while the more mature plant, as cut for hay, contained only 30 units.

By studying the carotene content of some South African feeds Myburgh (6) found that the carotene content of pasture plants diminished rapidly as the plants matured or as they became dry during winter or during periods of drought. His results on pasture grass during the four seasons for two years showed that the grass was relatively high in carotene in summer and autumn but rather low during the early spring and late winter.

In Arizona blue grama range grass lies in the dormant stage during the winter. In the summer a new growth is begun which is stimulated by rains. By the middle of October the grass begins to dry up again. Smith and Stanley (8) determined the vitamin A value of this grass at different stages of growth. They noted that a sample collected August 2 was a very potent

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source of vitamin A, by September 20 it was only one-half as rich, and when sampled in November, 100 times as much of the grass was required to produce the same rate of gain in rats as that cut in August.

Atkeson and colleagues (1) determined the carotene content of each of 13 pasture plants three or four times during the growing season, and observed that the plants were relatively high in carotene during the early summer, although there was considerable variation. Carotene decreased during the hot summer months but after the fall rains most plants reestablished their carotene content similar to early summer values. Big blue stem and buffalo grass were notable exceptions in that they showed a decrease as the season progressed.

Esselen and co-workers (2) found that the vitamin A content of the maize plant increased as the plant reached full growth after which there was a marked loss of vitamin A.

Watkins (11) studied the monthly variation in carotene content of black grama and mesa dropseed range grasses grown in New Mexico. His results indicate that rains which stimulate new growth also cause an increase in the carotene content of the grasses. Both grasses were moderately high in carotene during the growing season. The mesa dropseed lost all of its carotene soon after the fall freezes ended the growing season; however, the black grama grass, whose upright stems remained partly green throughout the winter contained an amount of carotene that seemed to be ample to satisfy the vitamin A requirements of range cattle.

#### EXPERIMENTAL

The purpose of this investigation was to study the weekly variation in the carotene content of various herbages throughout their growing season. The herbages studied included the following: a mixture of the Grimm and Harrison varieties of alfalfa, Canadian brome grass, corn leaves from Michigan hybrid corn 561, Iagold variety of oats, Manchu variety of soybeans, biennial white sweet clover, and Sudan grass. These herbages were grown on soil of average fertility on the college farm, except the sweet clover which was growing voluntarily on a vacant lot near the college. The alfalfa and brome grass were taken from a field with a good stand of an alfalfa-brome mixture. Samples for carotene analysis were collected once a week from early growth until the crop was harvested or cut for hay. Plants of average size were selected at random and their height ascertained. The appearance of blossoms or development of seeds was also noted at the time of sampling, so that the stage of maturity could be determined. The plants were cut off about an inch above the ground, wrapped in heavy paper and immediately taken to the laboratory.

In the laboratory, a representative portion of the plants was ground through a food chopper and thoroughly mixed in order to obtain a represen-

tative sample. A two to five gram sample was quickly weighed out and immediately covered with alcoholic KOH in a mortar. The sample was placed in the alcoholic KOH as soon as possible to prevent enzymatic or oxidative destruction of carotene. A small amount of fine clear quartz sand was added and the tissue thoroughly ground. The ground sample was then transferred to a 250 ml. Erlenmeyer flask, using sufficient alcoholic KOH to make the transfer complete.

The method used for extracting carotene from the plant tissue was the Guilbert method (3) as modified by Peterson, Hughes and Freeman (7). The carotene concentration of the extract was determined by means of a photo-electric colorimeter. The moisture content of all samples was also determined in order to calculate the results in terms of micrograms of carotene per gram of dry matter. For the moisture determination, five grams of the ground tissue were weighed out and dried for three hours in a drying oven at 100° C.

#### RESULTS

Data are presented in table 1 showing the weekly variation in the carotene content of the herbages studied during their growing season of 1938. The data include the results for three crops of alfalfa, two crops of brome grass, corn leaves during the latter part of growth, one crop of oats, one crop of soybeans, two crops of Sudan grass, and one crop of sweet clover.

The first sample of alfalfa was collected April 25 at which time it contained 432 micrograms of carotene per gram of dry matter and when it was cut for hay June 20 this value was only 171 micrograms. The decrease in carotene was not gradual and progressive for the first crop. Sampling of the second growth was begun 17 days after the first crop was cut for hay at which time the carotene content was 318 micrograms. This value dropped progressively to 132 micrograms by August 16 when the second growth was cut for hay. The carotene content of the first sample of the third crop was 367 micrograms, and five weeks later when in the three-fourths bloom stage this value was still 284 micrograms. The third crop showed a gradual decrease in carotene as the plants matured except for one sample taken September 21 at which time the value was slightly higher than that for the previous week.

Sampling of the brome grass was begun April 25 at which time it contained 453 micrograms of carotene per gram of dry matter. When the grass was cut for hay June 6, the carotene content was only 141 micrograms. Fifteen days later the second growth reached a height of seven inches and the carotene content was 466 micrograms. The carotene increased to 518 micrograms by the following week, but thereafter decreased. When the second growth was cut for hay the carotene content was still 317 micrograms. This value was more than twice the value of the first crop when cut for hay.

The first sample of corn leaves was collected as the tassels appeared on the corn, and at that time the carotene content was 482 micrograms per gram

TABLE 1

*Carotene content of several herbages in micrograms per gram of dry matter, during their growing season*

Date	Height inches	Dry matter per cent	Carotene micrograms	Remarks
Alfalfa—first crop				
4-25-38 .....	5	20.8	432.0	
5- 3-38 .....	8	20.4	397.0	
5- 9-38 .....	11	22.9	269.0	
5-16-38 .....	13	24.2	278.0	
5-23-38 .....	18	19.0	295.0	
5-29-38 .....	23	20.0	233.0	raining
6- 6-38 .....	24	22.0	188.0	"
6-13-38 .....	28	25.7	150.0	few blossoms
6-20-38 .....	31	27.1	171.0	early bloom
6-20-38 .....				half bloom
				cut for hay
Alfalfa—second crop				
7- 7-38 .....	11	19.4	224.6	
7-11-38 .....	13	19.1	301.9	
7-18-38 .....	17	26.7	272.7	few blossoms
7-26-38 .....	20	32.3	221.5	early bloom
8- 2-38 .....	21	32.4	212.8	half bloom
8- 9-38 .....	22	32.9	169.9	1/2 bloom
8-16-38 .....	22	31.8	116.0	1/2 bloom
8-17-38 .....				cut for hay
Alfalfa—third crop				
8-23-38 .....	15	21.1	366.7	
8-29-38 .....	18	21.6	368.2	
9- 5-38 .....	20	24.6	340.6	
9-13-38 .....	21	28.8	311.7	few blossoms
9-21-38 .....	22	27.5	321.1	early bloom
9-26-38 .....	22	29.9	283.6	half bloom
				1/2 bloom
Brome grass—first crop				
4-25-38 .....	3	23.8	453.0	
5- 2-38 .....	5	22.8	377.0	
5- 9-38 .....	11	23.6	284.0	
5-16-38 .....	15	22.5	311.0	
5-23-38 .....	21	19.8	293.0	
5-29-38 .....	26	17.1	306.0	raining
6- 6-38 .....	34	22.0	157.0	"
6-13-38 .....	36	30.2	144.8	few heads out
6-20-38 .....	45	32.0	140.6	heads out
				cut for hay
Brome grass—second crop				
7- 5-38 .....	7	15.1	466.0	
7-11-38 .....	10	13.8	517.6	
7-18-38 .....	15	18.0	418.5	
7-25-38 .....	19	24.7	412.9	
8- 2-38 .....	20	25.5	390.8	
8- 9-38 .....	23	29.6	305.2	
8-16-38 .....	23	24.4	317.1	
				few heads
				raining
				cut for hay

TABLE 1.—(Continued)

Date	Height inches	Dry matter per cent	Carotene micrograms	Remarks
Corn leaves				
8- 9-38 .....	105	25.4	481.6	tassels out
8-17-38 .....	110	22.9	615.7	pollen ripe
8-24-38 .....	112	27.5	646.1	early milk
8-30-38 .....	115	25.6	561.2	early milk
9- 5-38 .....	118	29.8	539.1	late milk
9-13-38 .....	118	24.2	516.5	dough stage
9-21-38 .....	118	27.7	361.0	glaze stage
9-26-38 .....	116	31.3	243.3	grain hard
10- 3-38 .....	118	59.4	68.4	corn ripe
10-11-38 .....	118	90.0	25.4	leaves dry
Oat plant				
5-11-38 .....	6	14.3	525.0	
5-18-38 .....	8	14.9	442.9	
5-25-38 .....	10	14.7	449.0	
6- 1-38 .....	15	14.0	375.0	
6- 8-38 .....	18	16.4	219.5	
6-15-38 .....	20	17.5	260.0	
6-22-38 .....	30	19.8	140.5	head in boot
6-29-38 .....	34	24.5	100.4	head out of boot
7- 7-38 .....	39	29.4	66.1	milk stage
7-11-38 .....	39	30.7	54.3	dough stage
7-18-38 .....	39	40.0	14.7	late dough
7-25-38 .....	39	48.7	12.6	ripe
				harvested
Soybean plant				
6-29-38 .....	9	22.5	279.0	
7- 5-38 .....	14	20.3	288.8	few blossoms
7-12-38 .....	17	21.3	299.5	early bloom
7-19-38 .....	21	22.5	357.8	few pods
7-26-38 .....	22	22.6	432.2	more pods
8- 2-38 .....	27	26.4	406.6	few beans
8- 9-38 .....	30	25.3	334.7	small beans
8-16-38 .....	34	24.5	248.7	beans developing
8-23-38 .....	36	29.0	196.9	" "
8-29-38 .....	36	29.5	193.6	" "
9- 5-38 .....	36	30.3	163.4	beans developed
9-13-38 .....	36	34.5	30.5	yellow leaves
9-21-38 .....	37	34.5	35.4	beans mature
9-26-38 .....	36	38.4	21.0	leaves dropping
10- 3-38 .....	36	66.8	7.9	stalks dry
Sudan grass—first crop				
7- 7-38 .....	18	15.6	454.7	
7-12-38 .....	34	17.1	316.9	
7-19-38 .....	43	20.4	218.5	
7-26-38 .....	48	25.0	198.4	heading
7-29-38 .....				grass cut
Sudan grass—second crop				
8- 9-38 .....	10	20.1	362.1	
8-17-38 .....	20	15.8	403.8	raining
8-24-38 .....	36	17.9	317.9	heads in boot
8-30-38 .....	43	18.4	229.5	heads out of boot
9- 5-38 .....	46	24.0	179.3	seeds developed
9-21-38 .....	48	25.3	112.5	leaves yellow
9-26-38 .....	50	36.1	57.6	grass cut

TABLE 1.—(Continued)

Date	Height inches	Dry matter per cent	Carotene micrograms	Remarks
Sweet clover				
4-27-38 .....	7	14.6	218.0	
5- 4-38 .....	12	12.8	242.0	
5-11-38 .....	18	16.4	244.0	
5-18-38 .....	20	16.0	175.0	
5-25-38 .....	24	16.0	216.0	
6- 1-38 .....	30	17.4	208.0	
6- 8-38 .....	37	19.2	177.0	few blossoms
6-15-38 .....	42	20.0	172.0	early bloom
6-22-38 .....	66	22.8	125.0	full bloom
6-23-38 .....				cut

of dry matter. This value increased during the next two weeks to 646 micrograms, after which time there was a progressive decrease in carotene as the plants matured. When the corn was mature enough for husking the carotene content of the dried leaves was only 25 micrograms.

When the oats were six inches high sampling was begun. At this time the carotene content was 525 micrograms per gram of dry matter, and when harvested this value had decreased to 13 micrograms. The results showed a definite downward trend in carotene content as the plants matured, although the decrease was not uniform from week to week. The most notable drop in carotene was during the early growing season until the heads appeared, during which time the original 525 microgram value was lowered to 100 micrograms.

The first values for soybeans were obtained when the plants were nine inches high, at which time the carotene content was 279 micrograms per gram of dry matter. This value gradually increased to 432 micrograms when the plants were 22 inches high and the bean pods were developing. From this stage of maturity on, there was a gradual and progressive decrease in carotene as the plants matured, so that by the time the leaves were yellow the carotene content of the plant was only 35 micrograms.

The carotene analyses for sweet clover are also presented in table 1. The carotene content of the young plants increased progressively to a rather low maximum of 244 micrograms per gram of dry matter when the plants were 18 inches high. After that there was a decrease as the plants matured and when in full bloom the carotene content was only 126 micrograms.

#### DISCUSSION OF RESULTS

The results for the herbages studied clearly show that the carotene content per gram of dry matter is much higher when the plants are in the earlier stages of growth than after they reach the stage of maturity at which they are usually harvested. The early growth alfalfa contained more than 300 micrograms of carotene per gram of dry matter, but when cut for hay, the

first crop contained only 171 micrograms and the second crop 133 micrograms. The carotene content of the third crop was considerably higher. However, the samples were taken from a different field so the results may not be directly comparable. Soybeans, on the other hand, when 10 to 15 inches high, contained slightly less than 300 micrograms of carotene per gram of dry matter but this value increased to 432 micrograms when the plants were 22 inches high and by the time the beans were developing, at which stage they are usually cut for hay, this value was approximately 200 micrograms.

First crop brome grass contained 141 micrograms of carotene per gram of dry matter when cut for hay, while the second crop contained 317 micrograms. This difference was probably due to the fact that the first crop developed to a more mature stage than did the second. The carotene content of Sudan grass, 18 to 20 inches high, was slightly more than 400 micrograms, but after heading this value dropped to about 200 micrograms. Both crops followed the same rate of decrease. The oat plant when 6 to 10 inches high contained more than 400 micrograms but after heading this value dropped to less than 150 micrograms. The sweet clover used in this investigation was lower in carotene than the other plants studied. The maximum of 244 micrograms per gram of dry matter was noted at 18 inches, which value decreased to 125 micrograms when in full bloom. On the other hand, corn leaves reached a maximum of 646 micrograms when the ears were in early milk stage, which value decreased to 517 micrograms when in the dough stage. As the leaves became yellow the carotene decreased very rapidly to a low value of 25 micrograms.

The decrease in carotene content as the plants matured was not progressive in all cases. During the latter part of May, alfalfa and brome grass, especially, did not show an even rate of decrease. As a matter of fact, in some cases a slight increase in the trend was noted. This was probably due to an increase in the rate of growth of the plants caused by the additional rainfall during that time. A report by the U. S. Weather Bureau revealed that the total precipitation for the month was 5.73 inches, most of which fell during the latter half of the month. This additional rainfall apparently increased the rate of growth which is concomitant with an increased carotene content (10, 11).

Some of the variations in carotene content during the growing season cannot be explained on the basis of differences in the rate of growth. Attention should be called to the fact that the carotene analyses were made on different plants in successive weeks. Thus some of the variations from week to week may have been due entirely to sampling, since it is almost impossible to select random samples under normal growing conditions that are exactly comparable. Most of the carotene of herbages is contained in the leaves (4). Thus in the event of selecting a sample for analysis which contained more stems, the carotene content would be lower, while a plant with more leaves would

contain more carotene. This, and also the fact that plants are biological tissue in which changes are continually taking place, might help explain some of the variations from the normal trends.

The differences in trends for the carotene content of the various plants during growth were interesting. In the case of alfalfa, brome grass, oats, and Sudan grass, the carotene content decreased from the earliest samples until maturity. The second growth of brome grass did show an increase during the first week, but thereafter the regular decrease was noted. The sweet clover showed a gradual increase during the first two weeks after which the trend was downward. In contrast to these plants the soybeans showed a progressive increase in carotene up to and including three weeks' growth after the first blossoms appeared, but thereafter a definite downward trend was noted. In the case of corn leaves, which were used in this investigation, rather than the entire plant, sampling was not begun until the tassels were out, at which time the carotene content was increasing. The increase continued for two weeks, after which a definite and progressive decrease was noted. These variations in trends may be due entirely to plant species differences. It should be mentioned, however, that the blooming process in the case of soybeans is slow and gradual.

From the results obtained it would seem evident that plants should be cut at an early stage of growth in order to obtain the greatest carotene content. However, for ensilage the crop should be cut at that stage of maturity which is complementary to proper preservation in the silo.

#### SUMMARY AND CONCLUSIONS

1. Variations in the carotene content of seven herbages were studied during their growing season. These herbages included alfalfa, brome grass, corn leaves, the oat plant, the soybean plant, Sudan grass, and sweet clover.
2. The carotene content of these herbages is much greater during the earlier stages of growth than after they reach the stage of maturity at which they are usually harvested.
3. The carotene content, when calculated in terms of micrograms per gram of dry matter shows a rather progressive decrease as the plants mature except where affected by factors governing the rate of growth.
4. In making hay or silage, in order to obtain the greatest carotene content the plants should be cut at an early stage of maturity.

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NOTE: Since this paper was prepared three papers by F. E. Moon, dealing with similar subject matter, have appeared and should be noted. They are: 1, The composition of grass at various stages of maturity, and the changes occurring during haymaking, with particular reference to carotene content. *The Empire Journal of Exp. Agr.* 7: 225-234, 1939. 2, The carotene content of some grass and clover species, with a note on pasture weeds. *The Empire Journal of Exp. Agr.* 7: 235-243, 1939. 3, The influence of manurial treatment on the carotene content of poor pasture grass and on the relationship of this constituent to the ash and organic fractions. *Journal of Agr. Sc.* 29: 524-543. 1939.



ASCORBIC ACID AND OXIDIZED FLAVOR IN MILK. II. THE  
EFFECT OF VARIOUS HEAT TREATMENTS OF MILK  
UPON THE STABILITY OF ASCORBIC ACID  
AND UPON THE DEVELOPMENT OF  
THE OXIDIZED FLAVOR<sup>1</sup>

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The stability of ascorbic acid in milk seems especially important inasmuch as normal milk is not an abundant source of this vitamin. Being a reducing agent the presence of ascorbic acid influences oxidative changes in milk and thus has a bearing upon milk flavor. Within recent years many data have been presented showing factors which affect the ascorbic acid of milk and which affect the development of the oxidized flavor.

Kende (8) demonstrated that heating milk to 85° C. (185° F.) for five minutes inhibited the development of the oxidized flavor while pasteurization at 63° C. (145.4° F.) for 30 minutes was ineffective. He observed that 0.05–0.10 mg. of CuSO<sub>4</sub> per liter was sufficient to cause the oxidized flavor in raw milk or milk pasteurized by the low-temperature holder process, while 24–40 times that quantity had no effect on milk pasteurized at the high temperature. His studies led him to conclude that milk highly susceptible to oxidation would oxidize only in the presence of an external oxidative agent (*e.g.*, metal salts, copper particularly) and these acting only in the presence of an organic ferment, which he named "oleinase." The high-temperature exposure inactivated the "oleinase" thus inhibiting the development of the oxidized flavor.

With some modification as to the time and temperature of heating, Guthrie and Brueckner (6), Greenbank (5), Thurston (12), Chilson (2), Sharp, Trout, and Guthrie (10), Dahle and Palmer (3), and Gould and Sommer (4) noted also the inhibitory effect of high heat treatments of milk on the development of the oxidized flavor. Brown, Thurston, and Dustman (1) showed that the time of copper contamination with respect to the pasteurization exposure was an important factor in the development of the oxidized flavor.

Chilson (2) added reducing agents, ascorbic acid, elone and hydroquinone to milk and noted that their presence in sufficient quantities prevented the development of the oxidized flavor, for seven days, in milk which

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<sup>2</sup> The data presented in this paper are from a thesis submitted to the Graduate School of the Michigan State College in partial fulfillment of the degree of Master of Science, June, 1938.

ordinarily developed a very bad oxidized flavor within three days. He noted also that the addition of ascorbic acid to milk gave it a pleasing flavor, seemingly better than the original milk before it became oxidized. Fortified milk having 92.6 mg. of ascorbic acid per liter had only 85.2 mg. per liter when pasteurized at 143° F. (61.6° C.) for 30 minutes, whereas raw milk fortified with 150 mg. of ascorbic acid per liter retained all the ascorbic acid upon heating to 200° F. (93.3° C.) for a similar period.

King and Waugh (9), Whitnah, Riddell, and Caulfield (13), Sharp, Trout, and Guthrie (10), and Dahle and Palmer (3) observed less destruction of ascorbic acid when milk was heated out of contact with copper at temperatures of 160° F. (71.1° C.) or above than when holder pasteurized at 143–145° F. (61.6–62.8° C.).

Hand, Guthrie, and Sharp (7) demonstrated that holder pasteurized milk voided of oxygen by vacuum cooling neither decreased the ascorbic acid content nor developed the oxidized flavor after holding seven days although 0.1 mg. copper per liter was added. In fact, pasteurized milk subjected to the vacuum cooling treatment was much higher in ascorbic acid after holding than the raw milk held for the same length of time.

Gould and Sonnmer (4), studying the effect of heat on milk with special attention to the cooked flavor, found that the cooked flavor of milk was caused by the formation of sulphides which occurred when milk was subjected to sufficiently high temperatures or to other changes occurring simultaneous to this formation. They found that temperatures sufficiently high to cause a cooked flavor also largely inhibited the development of the oxidized flavor. However, when the addition of copper followed the heating process, no relationship between the cooked and the oxidized flavor was observed. They believed that in addition to being responsible for the cooked flavor of milk, the presence of these sulphides might explain the heat retardation effect resulting in the prevention of the development of the oxidized flavor, an effect attributed by Kende (6) to be due to inactivation of the organic ferment which he designated "oleinase."

#### EXPERIMENTAL

The milk used in these studies was machine-drawn into an aluminum container from which it was poured into glass bottles and cooled immediately. The samples were processed in glass, using laboratory equipment. The holding exposures employed were 63° C. (145.4° F.) and 75° C. (167° F.) for 30 minutes and 65 (149), 70 (158), 75 (167), 80 (176), 85 (185), and 90° C. (194° F.) for ten minutes. Samples were flash pasteurized also at ranges of temperature from 60–97° C. (140–206.6° F.) for five to fifteen seconds by drawing the milk, using low vacuum, through glass tubing submerged in hot and cold water baths for appropriate heating and cooling. Thermometers placed in the line of flow indicated the desired

temperatures. The milk, stored at 5° C. (41° F.), was titrated daily for ascorbic acid, titrations being made from portions of each sample using 2-6 dichlorophenolindophenol according to the rapid method of Sharp (1).

Copper was added to the milk in varying amounts before or after pasteurization as the experiment dictated.

After sufficient storage the milk was examined organoleptically for flavor.

For experiment 1, individual samples of milk were obtained from 20 cows of the college herd and were cooled and treated at once. Each sample was divided into five lots: one lot served as a control; two lots were pasteurized at 63° C. (145.4° F.) for thirty minutes, after which 0.13 mg. of copper per liter was added to one lot; the two remaining lots were pasteurized at 75° C. (167° F.) for thirty minutes, after which copper was added to one lot as above.

In experiment 2, samples of milk from six individual cows were flash pasteurized at various temperatures from 60-97° C. (140-206.6° F.) and cooled within a period ranging from five to fifteen seconds.

The milk used in experiment 3 was obtained from three cows, numbers 30, 111, and 116, two Jerseys and a Guernsey, respectively. The milk was divided into two groups, I and II, of six lots each, A, B, C, D, E, and F. The lots were again divided into six portions, 1, 2, 3, 4, 5, and 6 of 200 ml. each. To the six 200 ml. portions copper was added at the rate of 0, 0.13, 0.26, 0.39, 0.52, and 0.65 mg. per liter, respectively. The copper was added to the milk comprising Group I before heating and to that of Group II after heating. Lots A, B, C, D, E, and F of both the Groups I and II were heated for exactly ten minutes at 65 (149), 70 (158), 75 (167), 80 (176), 85 (185), and 90° C. (194° F.), respectively. The milk was treated and the ascorbic acid value determined the same day as milking as well as after various storage periods.

#### RESULTS

1. *The effect of copper upon the stability of ascorbic acid and upon the development of the oxidized flavor when the milk was pasteurized for 30 minutes at high and at low temperature.*

The catalyzing effect of copper upon the oxidation of ascorbic acid and upon the oxidation of the fatty constituents of milk was studied in individual samples of milk from 20 cows of the college herd.

The data obtained are presented in tables 1 and 2, and are shown graphically in figure 1.

An examination of the data of table 1 shows the catalyzing effect of copper upon the oxidation of ascorbic acid when the milk was pasteurized at 63° C. (145.4° F.) for thirty minutes. This effect was very marked after the second and third days. On the fourth day no appreciable amount of

TABLE I  
The ascorbic acid content and the development of the oxidized flavor in milk from individual cows when the milk was holder pasteurized, had copper added, and was stored at 5° C. (41° F.), May, 1937

Cow No.	Raw milk				Holder pasteurized milk (63° C. (145.4° F.)—30 min.)								
	1st day (control)	No copper added			Copper added (0.13 mg. per liter)								
		2nd day	3rd day	4th day	Oxidized flavor on 4th day	2nd day	3rd day	4th day	Oxidized flavor on 4th day	2nd day	3rd day	4th day	Oxidized flavor on 4th day
13	16.4	12.5	6.8	2.9	-	10.5	9.3	5.9	-	2.0	1.9	0.0	-
30	23.6	22.6	19.6	16.7	-	22.0	19.7	12.7	-	16.0	6.8	2.5	-
36	18.3	11.5	2.5	2.9	-	9.0	2.9	1.9	-	3.5	2.5	0.0	+
37	18.3	10.5	3.4	2.5	-	9.0	3.9	1.9	+	3.5	3.4	0.0	-
41	19.3	18.5	13.7	8.8	-	17.5	14.7	10.3	-	11.5	2.5	0.0	++
42	21.7	18.1	6.8	2.5	-	14.0	8.8	5.9	-	7.5	1.9	0.0	++
100	24.1	20.1	12.8	5.9	-	20.0	14.7	12.8	-	10.0	1.9	0.0	-
111	24.5	23.5	23.1	17.7	+	18.5	18.7	18.7	+	16.0	5.9	2.9	++
116	25.5	24.1	24.1	14.7	-	23.0	20.6	18.1	-	17.5	12.1	3.4	+
117	21.2	19.5	12.8	7.8	+	19.5	16.7	8.8	+	12.0	2.9	0.0	++
134	20.2	17.0	10.8	4.9	-	14.0	10.8	6.8	-	5.5	1.5	0.0	++
142	18.8	16.0	8.8	2.9	-	16.0	10.3	5.9	-	5.5	1.9	0.0	-
174	16.4	12.5	7.8	1.9	-	11.0	5.4	2.4	-	2.5	1.9	0.0	-
180	20.7	16.0	10.8	3.9	-	18.0	12.3	9.8	-	4.5	1.9	0.0	++
190	21.2	17.5	12.7	8.8	-	17.5	13.3	11.3	-	8.0	0.9	0.0	++
197	22.2	17.5	11.8	5.9	-	18.5	13.7	10.8	-	4.5	0.9	0.0	++
237	19.7	13.0	2.5	1.9	+	10.5	2.9	2.9	+	3.0	0.9	0.0	+
238	22.2	17.5	13.7	5.9	-	17.0	12.9	10.8	-	7.0	1.9	0.0	++
239	21.7	16.5	6.8	1.4	-	16.0	7.8	3.9	-	5.0	0.9	0.0	+
300	18.8	16.5	13.7	3.9	-	16.5	14.7	11.8	-	9.0	0.9	0.0	++
Avg.	20.7	17.1	11.3	6.2	-	15.9	11.7	8.7	-	7.7	2.8	0.4	-

\* Key: - no oxidized flavor.  
† doubtful oxidized flavor.

+ slight oxidized flavor.  
++ distinct oxidized flavor.  
+++ very strong oxidized flavor.

TABLE 2

The ascorbic acid content and the development of the oxidized flavor in milk from individual cows when the milk was heat treated at 75° C. (167° F.) for 30 minutes, had copper added, and was stored at 5° C. (41° F.), May, 1937

Cow No.	Raw milk				Milk heated to 75° C. (167° F.) and held 30 minutes								
					No copper added			Copper added (0.13 mg. per liter)					
	1st day (control)	2nd day	3rd day	4th day	Oxidized flavor on 4th day	2nd day	3rd day	4th day	Oxidized flavor on 4th day	2nd day	3rd day	4th day	Oxidized flavor on 4th day
13	16.4	12.5	6.8	2.9	-	13.5	11.3	10.3	-	12.0	11.3	8.8	-
30	23.6	22.6	19.6	16.7	-	19.0	18.2	16.2	-	19.5	16.7	14.7	-
36	18.3	11.5	2.5	2.9	-†	11.0	9.3	8.3	-	11.0	7.3	4.9	-
37	18.3	10.5	3.4	2.5	-	12.5	10.8	9.3	-	10.5	8.8	5.4	-
41	19.3	18.5	13.7	9.8	-	15.5	12.8	12.8	-	13.5	13.3	12.8	-
42	21.7	18.1	6.8	2.5	-	14.5	13.7	11.8	-	-	-	-	-
100	24.1	20.1	12.8	5.9	-†	17.0	16.2	14.7	-	17.0	14.7	14.3	-
111	24.5	23.5	23.1	17.7	+‡	18.5	18.7	16.7	-	18.5	17.2	16.7	-
116	25.5	24.1	24.1	14.7	-	18.0	17.2	14.7	-	19.0	17.7	17.7	-
117	21.2	19.5	12.8	7.8	+‡	17.0	13.7	11.8	-	17.0	14.3	14.7	-
134	20.2	17.0	10.8	4.9	-	15.0	12.8	10.3	-	13.0	11.8	6.4	-
142	18.8	16.0	8.8	5.9	-	11.0	10.3	10.3	-	14.0	10.8	7.8	-
174	16.4	12.5	7.8	1.9	-	12.5	10.8	10.3	-	11.0	8.8	6.4	-
180	20.7	16.0	10.8	3.9	-	14.5	14.3	13.2	-	14.0	12.8	9.8	-
190	21.2	17.5	12.7	8.8	-	17.0	14.3	13.7	-	14.5	11.8	7.8	-
197	22.2	17.5	11.3	5.9	-	14.5	14.3	12.8	-	16.0	14.3	14.3	-
237	19.7	13.0	9.5	1.9	-†	13.5	11.8	9.3	-	13.0	9.8	8.8	-
238	22.2	17.5	13.7	5.9	-	15.5	14.3	12.8	-	15.5	14.7	13.3	-
239	21.7	16.5	6.8	1.4	-	16.0	14.3	12.2	-	15.5	14.7	12.8	-
300	18.8	16.5	13.7	3.9	-	12.5	12.8	10.8	-	15.0	13.3	10.8	-
Avg.	20.7	17.1	11.3	6.2	-	14.9	13.6	12.1	-	14.7	12.9	10.9	-

\* Key: - no oxidized flavor.  
† doubtful oxidized flavor.

+ slight oxidized flavor.  
++ distinct oxidized flavor.

+++ very strong oxidized flavor.

ascorbic acid remained. Coincident with the marked decrease in ascorbic acid, the strong development of oxidized flavor was usually noted. However, this observation was not consistent in all samples. Some samples, pasteurized at 63° C. (145.4° F.) and treated with copper, exhibited a very pronounced decrease in the ascorbic acid content without any, or possibly a slight, development of the oxidized flavor. This was observed in milk from cows number 13, 36, 37, 100, 116, 174, 237, and 239. Of these, milk from two cows, number 13 and 100, developed no noticeable trace of oxidized flavor even when contaminated with 0.13 mg. of copper per liter after pasteurization.

The stability of ascorbic acid was slightly less on the average in raw milk over a three-day storage period than similar milk pasteurized by the holder method. A slightly greater loss of ascorbic acid was noted in the pasteurized milk than in the raw milk on the first day of storage. However, on the second day the average losses were about equal. The stability of the ascorbic acid as a result of the high heat treatment was greatly increased over that when the low temperature was employed. Nevertheless, there was a continuous slight decrease in the amount of ascorbic acid present during storage as shown in table 2 and figure 1.

The increased stability of ascorbic acid at the 75° C. (167° F.) exposure over that of the 63° C. (145.4° F.) exposure was shown more strikingly in the cases where copper was added to the milk. The presence of copper resulted in a slight decrease in the ascorbic acid of the milk similarly high

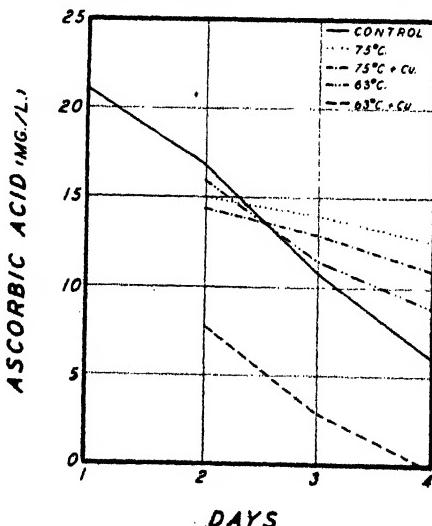


FIG. 1. The relative stability of ascorbic acid in milk pasteurized for 30 minutes at 63° C. (145.4° F.) and at 75° C. (167° F.), respectively, with and without the addition of 0.13 mg. per liter of copper following pasteurization.

heat treated without copper, but was comparatively stable compared to that in milk pasteurized at the lower temperature and held under similar conditions. Oxidized flavor did not develop in the samples thus high heat treated even in the presence of 0.13 mg. of copper per liter.

An examination of tables 1 and 2, shows that milk from certain cows exhibited a natural stability in respect to ascorbic acid, particularly when untreated. This stability was especially noticeable in the case of cows number 30, 111, 116, two Jerseys and a Guernsey, respectively. An examination of the oxidized flavor data showed that the pasteurized copper contaminated milk from these cows was susceptible also to the development of the oxidized flavor. In fact, milk from cow number 111 had already shown, while raw, a tendency to develop the oxidized flavor.

## 2. The effect of flash heating of milk at various temperatures upon the stability of the ascorbic acid.

Individual samples of milk from 10 cows were flash pasteurized at various temperatures from 60–97° C. (140–206.6° F.) and cooled within a period ranging from 5 to 15 seconds. Portions of each sample were titrated daily for seven days for ascorbic acid.

An examination of the data obtained shows that the stability of ascorbic acid in milk from individual cows was not entirely the same under similar conditions of heat treatment; that flash pasteurization temperatures ranging from 85–95° C. (185–203° F.) had the most pronounced stabilizing effect

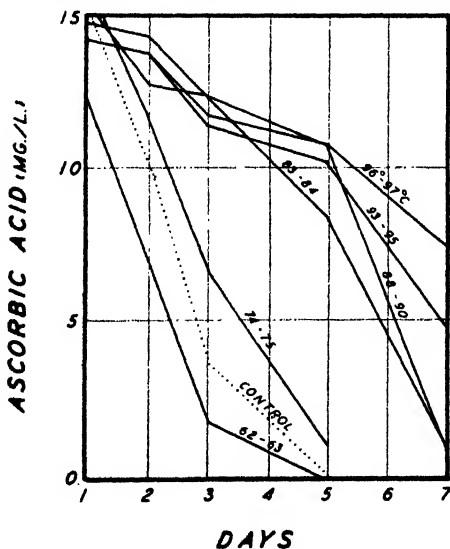


FIG. 2. The effects of various flash pasteurization temperatures ( $^{\circ}$  C.) upon the ascorbic acid content of milk from cow number 144 when stored for several days, June 1937.

upon the ascorbic acid; that flash exposures below 75° C. (167° F.) had slightly less stabilizing effect upon the ascorbic acid than that existing naturally in the raw milk; that the critical flash exposure minima for ascorbic acid stability were between 75 and 85° C. (167 and 185° F.); and that milk having a naturally low ascorbic acid content usually showed greater increased stability of ascorbic acid upon flash heating than did those samples having a naturally high ascorbic acid value.

Data obtained on the milk from two cows which seemed representative of those studied, are shown graphically in figures 2 and 3.

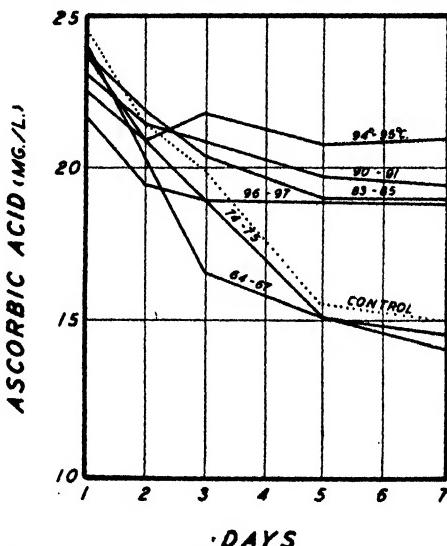


FIG. 3. The effects of various flash pasteurization temperatures (°C.) upon the ascorbic acid content of milk from cow number 116 when stored for several days, June 1937.

3. *The effect of ten-minute exposures at various temperatures upon the ascorbic acid content of milk and upon the development of the oxidized flavor when copper was added before and after heating.*

Mixed milk from cows number 30, 111, and 116 of the college herd was subjected to ten minute exposures at various temperatures. Copper was added to the milk before and after heating. The ascorbic acid values obtained on the milk with the various treatments, initially and after 24 hours storage, are presented graphically in figures 4 and 5.

A temperature of 80° C. (176° F.) for 10 minutes seemed to be an exposure during which marked changes took place in milk as far as factors affecting the stability of ascorbic acid were concerned. When the copper was added to the milk *before* heating, the optimum temperature for the stability of ascorbic acid, regardless of the different amounts of copper

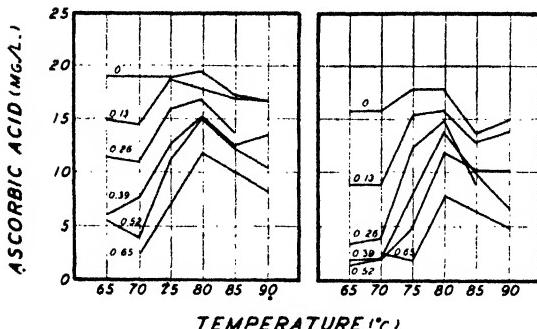


FIG. 4. The stability of ascorbic acid in milk when heated to various temperatures for ten minutes. The milk was contaminated with various amounts of copper (mg. per liter) before heating. Values at initial titration and after 24 hours storage respectively.

added, was found to be 80° C. (176° F.). With the addition of increasing amounts of copper, the ascorbic acid content became less regardless of exposure, but 80° C. (176° F.) remained, nevertheless, the optimum temperature of ascorbic acid stability for each individual series. At exposures above 80° C. (176° F.) the ascorbic acid was less stable. However, greater stability was exhibited in the milk heated above 80° C. (176° F.) for ten minutes than when heated to temperatures below 80° C. (176° F.).

When the copper was added to the milk *after* heating, the maximum stability of ascorbic acid was noted at the 80° C. (176° F.) exposure also. However, at the exposures of 80° C. (176° F.) and above the ascorbic acid remained comparatively stable for each amount of copper added, an observation not noted when the copper was added to the milk prior to heating.

The data showed that the development of oxidized flavor tended to follow the destruction of ascorbic acid. A marked decrease in the inten-

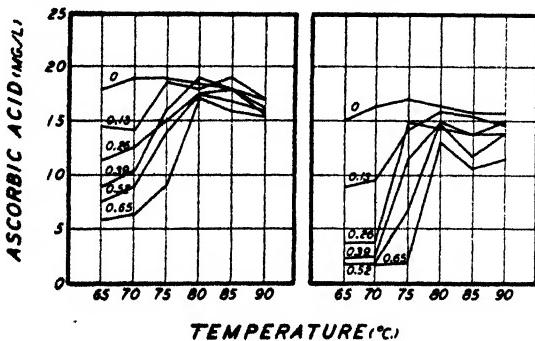


FIG. 5. The stability of ascorbic acid in milk when heated to various temperatures for ten minutes. The milk was contaminated with various amounts of copper (mg. per liter) after heating. Values at initial titration and after 24 hours storage.

sities of the oxidized flavor was noted when the milk was heated to 80° C. for ten minutes, the critical exposure noted in this experiment for the stability of ascorbic acid.

The time of adding copper with respect to the heat treatment was of importance also in the development of the oxidized flavor. When the copper was added to the milk after heating to 65 or 70° C. (149 or 158° F.) for ten minutes a distinct to a very strong oxidized flavor developed as contrasted to a slight oxidized flavor development when the copper was added before heating. At the 75 (167), 80 (176), and 85° C. (185° F.) exposures little difference was encountered in the intensities of the developed oxidized flavors, which required the presence of from 0.26 to 0.39 mg. of copper per liter for acceleration, regardless of its addition before or after heating. At the 90° C. (194° F.) exposure, however, the oxidized flavor was not noted in the milk contaminated with 0.52 or 0.65 mg. of copper per liter before heating, but was slight to distinct when similar amounts of copper were added after heating. This observation would seem to indicate that copper present in milk at the time of heating must have been combined in part or rendered unavailable for acceleration of the oxidation of the fatty constituents.

An important difference between the stability of ascorbic acid and the development of the oxidized flavor was noted. The ascorbic acid exhibited maximum stability at a definite exposure, 80° C. (176° F.) for ten minutes, whereas no specific exposure was noted to be effective under all conditions in preventing the oxidized flavor development. Several factors, such as the amount of copper added and the time of addition with respect to the heat treatment, were of importance in establishing an exposure which would inhibit the development of the oxidized flavor.

#### DISCUSSION AND SUMMARY

Individual samples of milk from 20 cows were pasteurized for 30 minutes at 63° C. (145.4° F.) and at 75° C. (167° F.) with and without the addition of 0.13 mg. of copper per liter. The ascorbic acid was quite unstable in the milk processed at 63° C. (145.4° F.) with copper added following pasteurization. Such milk was very prone to the development of the oxidized flavor upon three days storage. Exceptions, however, were noted. The ascorbic acid of some cows' milk seemed to exhibit a natural stability lacking in other samples. The rapid disappearance of the reduced form of ascorbic acid was not always an assurance that oxidized flavor would develop, inasmuch as some samples were found having no ascorbic acid after three days storage, yet, although copper was present, oxidized flavor did not develop.

When the milk was pasteurized at 75° C. (167° F.) for 30 minutes, the ascorbic acid exhibited marked stability with no development of oxidized

flavor after three days storage regardless of the presence of 0.13 mg. of copper per liter.

Flash pasteurization of samples of milk from individual cows at temperatures ranging from 60–97° C. (140–206.6° F.) for fifteen seconds or less had varying effects upon the stability of ascorbic acid. The critical flash exposure minima for ascorbic acid stability were between 75 and 85° C. (167 and 185° F.); exposures below having slightly less stabilizing effect and exposures above having a more pronounced stabilizing effect than that existing naturally in the raw milk.

Samples of mixed milk from three cows were heated to 65 (149), 70 (158), 75 (167), 80 (176), 85 (185), and 90° C. (194° F.) for ten minutes with and without the addition of various amounts of copper. A temperature of 80° C. (176° F.) for 10 minutes seemed to be an exposure which caused marked changes in milk as far as factors affecting the stability of ascorbic acid and the development of the oxidized flavor were concerned. Whether copper was added before or after heating, the 80° C. (176° F.) exposure had the greatest stabilizing effect. When the copper was added to the milk *after* heat treatment a marked stability of the ascorbic acid was noted in the samples heated to 80° C. (176° F.) and above. However, when the copper was added *before* heat treatment this same stability was not observed. Furthermore, the addition of various amounts of copper before heat treatment seemed to have a greater destructive effect on the ascorbic acid, even at 80° C. (176° F.), than when the copper was added after heat treatment.

As has been shown previously (1) the time of adding copper with respect to the heat treatment was of importance in the development of the oxidized flavor. When the milk was exposed to 65° C. (149° F.) or to 70° C. (158° F.) for ten minutes and then treated with copper a distinct to a very strong oxidized flavor developed as contrasted to a slight oxidized flavor development when the copper was added before heating. At the 75 (167), 80 (176), and 85° C. (185° F.) exposures little difference was encountered in the intensities of the developed oxidized flavor, which required the presence of 0.26 to 0.39 mg. of copper per liter to develop a distinct oxidized flavor, regardless of its addition before or after heating. At 90° C. (194° F.), however, the oxidized flavor was not noted in the milk contaminated with 0.52 or 0.65 mg. of copper per liter before heating, but developed slightly when similar amounts of copper were added after heating.

Many observations on the flavor of milk treated at high temperatures in this and in other studies have shown a lessened tendency for the development of the oxidized flavor at temperatures at which the cooked flavor occurred. If distinct oxidized flavors developed up to a certain temperature of heat treatment they were generally "pure" oxidized after which

they usually become "pure" cooked. The similarity between the most effective temperatures in stabilizing the ascorbic acid and in retarding the development of the oxidized flavor found in these studies and the temperatures at which liberation of sulphides and cooked flavor occurred, as recently reported by Gould and Sommer (4), leads to the supposition that the formation of reducing substances at the higher temperatures, may play an important role both in the ascorbic acid titration values and in the development of the oxidized flavor.

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# THE EFFECT OF FEEDING PEA VINE SILAGE ON THE CAROTENE AND VITAMIN A CONTENT OF MILK<sup>1,2</sup>

B. CONNOR JOHNSON AND W. H. PETERSON

Many farmers in pea canning areas have found that silage made from pea vines is a satisfactory feed for dairy cattle. A preliminary report was published last year (1) indicating that well-made pea vine silage may prove a valuable feeding material for improving the vitamin A potency of winter milk. A cooperative experiment was carried out in the winter of 1938-39 with Warren G. Clark of the Central Wisconsin Canneries at Beaver Dam, on the feeding of pea vine silage to his dairy herd. The experiment was primarily to determine the efficiency of this type of silage in the production of milk of a high carotene and vitamin A content. A group of Holstein cows was fed this silage, while at the same time other cows of the same herd were fed the same dry ration without the added silage. Carotene determinations were made on the silage, and carotene

TABLE I  
*Analysis of pea vine silage*

No.	Date	Treatment	Appearance and odor	Dry matter	pH	Carotene
Pea Vine Silages from the Warren G. Clark Farm						
2	December 16, 1938	H <sub>3</sub> PO <sub>4</sub>	Good	26.0	4.2	189
3	January 9, 1939	"	"	21.3	4.4	98
4	February 6	"	"	21.6	4.3	114
6	March 21	"	"	26.4	4.8	117
7	April 17	"	"	29.0	3.9	130
Pea Vine Silages from Other Sources*						
1	November 16, 1938	H <sub>3</sub> PO <sub>4</sub>	Good	23.5	3.10	163
2	" 15	A.I.V.	"	19.7	4.8	232
3	March 20, 1939	"	"	31.6	3.65	187
4	December 16, 1938	None	"	20.0	4.8	328
5	April 17, 1939	"	Poor odor	23.9	5.46	170
6	February 6, 1939	"	Good	21.8	4.5	172
7	January 12	"	Fair	20.2	5.1	50
8	" 12	"	Poor	18.8	5.1	7
9	December 16, 1938	Stack, none	Good	27.7	4.15	186
10	" 24	" "	"	23.0	4.32	184
11	March 20, 1939	" "	"	23.5	4.07	145
12	December 16, 1938	" "	"	30.1	4.45	120

\* These are silo silages, unless otherwise stated.

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<sup>2</sup> The authors are indebted to Warren G. Clark and Berger Sandstrom for their cooperation in planning and carrying out the feeding work of this paper.

and vitamin A determinations on the milk at regular intervals. Milk production records were also kept during the experiment.

This pea vine silage was prepared with 55 per cent phosphoric acid added at the rate of one gallon per ton. The acid was diluted 1 to 4 and put on the forage in layers, that is, pea vines were filled in for about one foot, diluted acid was poured over it; then another foot of pea vines was added and followed by acid, and in this way the silo was filled. This uneven addition of acid may explain the variations in carotene content. The carotene in the silage was determined by the method of Hegsted, Porter and Peterson (2). The results of the silage analysis are given in table 1. For comparative purposes, analysis of samples of pea vine silage of various types from other parts of Wisconsin are included in this table.

Twenty-two cows were maintained on a ration consisting of 15 pounds of good quality alfalfa hay, 10 pounds dry corn stalks, 13 pounds corn and cob meal and soybean hay (equal parts by weight) and 25 pounds pea vine silage per cow per day, for the 17 weeks of the experiment. After several weeks on this ration, three cows were changed to a ration in which

TABLE 2  
*Analysis of milks from cows fed pea vine silage*

Date	Carotene*	Vitamin A*	Total potency*	Ration**
Herd cows				
January 3, 1939	3.8	11.1	13.0	Pea vine silage
" 9	3.1	11.1	12.7	" " "
" 23	2.7	11.0	12.4	" " "
February 6	3.2	13.6	15.2	" " "
" 14	3.3	13.2	14.8	" " "
March 15	2.9	10.7	12.2	" " "
" 21	3.0	9.7	11.2	" " "
April 18	2.5	9.1	10.4	" " "
Experimental cows				
December 20, 1938	3.7	10.6	12.5	Pea vine silage
January 3, 1939	3.7	11.5	13.4	Silage omitted
" 9	3.4	10.6	12.3	" "
" 23	2.6	9.0	10.3	" "
February 6	2.5	8.7	10.0	" "
" 14	2.9	9.0	10.5	Silage begun again
" 28	3.7	10.2	12.1	continued
March 15	5.7	9.6	12.4	" "
" 21	2.5	10.7	12.0	" "
April 18	3.1	10.3	11.9	" "
Milk from other herds on pea vine silage				
				Source
December 16, 1938	7.1	12.4	15.9	Columbus
February 17, 1939	5.3	9.1	11.8	"
January 4, 1939	4.3	9.1	11.2	Rockmarsh

\* Expressed in micrograms per gram butterfat.

\*\* See Table 3 for complete ration.

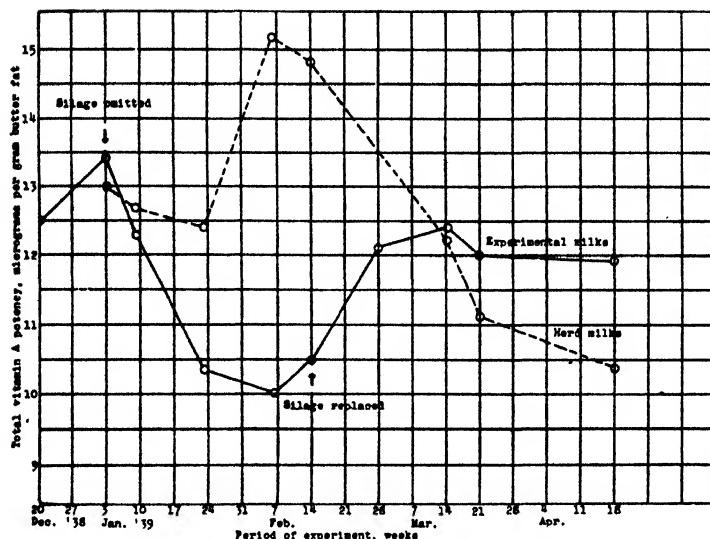


FIG. 1. Vitamin potency of herd and experimental milks.

the silage was replaced by alfalfa hay on an equivalent dry matter basis. They were kept on this dry ration for 6 weeks, at which time the silage was again placed in the ration.

TABLE 3  
*Milk production of experimental cows*

Date	Pounds of milk produced	Ration per cow
<i>Fore Period. Silage</i>		
December 20, 1938	55.7	Hay, alfalfa 15 lbs., pea vine silage, 25 lbs., corn stalks 10 lbs., corn and cob meal and soy bean hay 13 lbs.
December 27	53.0	Same
January 3, 1939	55.0	"
<i>Test Period. No silage</i>		
January 6, 1939	49.9	Same as fore period except pea silage replaced with alfalfa hay.
January 9	44.3	Same
January 17	45.0	"
January 25	46.5	"
February 7	43.0	"
February 15	41.5	"
<i>After Period. Silage</i>		
February 22	40.8	Same as fore period.
February 26	40.5	" " " "
March 14	42.0	" " " " *
March 21	39.3	" " " " *
April 17	36.7	" " " " *

\* Pea vine-sudan hay silage instead of pea vine silage.

The milk from both groups of cows was analyzed for carotene and vitamin A by the method of Olson, Hegsted and Peterson (3), at intervals throughout the experiment. The results are given in table 2 and plotted on figure 1. In table 2 are also given for comparison, the results from milks from other cows on pea vine silage. In figure 1 the curves represent the total vitamin A value of the milk. In making this calculation, carotene is taken as having a potency one-half that of vitamin A, weight for weight (4). The milk production for the three experimental cows is given in table 3.

#### DISCUSSION

Pea vine silage has been reported as worth 91 per cent as much as good corn silage for dairy cows (5). It was also pointed out that less protein supplements are needed when pea vine silage is fed, as this silage is considerably richer in protein than corn silage. Morrison (6) has given the analysis for nutrients of a sample of pea vine silage and has pointed out its value in live stock feeding.

From figure 1 it can be seen that the feeding of this pea vine silage at 25 pounds a day maintained the vitamin A and carotene at a high level. When the silage was omitted from the ration, the vitamin A value of the milk quickly decreased. On replacement of silage in the ration of the cows that had been on dry feed for six weeks, the vitamin A value of their milk rose again to approximately its former level, and a level equal to that of milk produced by the cows that had received silage throughout the experiment. This shows definitely that the silage was responsible for the high vitamin A value of this milk. Comparison shows these values to be much higher than the average values of winter market milks in this state. These were found by Dornbush *et al.* (7) for January 1939 to April 1939 to average approximately 8.8 micrograms per gram butter fat.

Also from table 3 the rate of decrease of milk production is found to be more rapid during the period when the cows did not receive silage. There was a decrease of about 0.4 pound per week in the fore period (two weeks), 2.2 pounds per week in the experimental period (six weeks) and 0.6 pound per week in the after period (eight weeks).

#### SUMMARY

Cows fed a good quality of pea vine silage produced a milk higher in carotene and vitamin A than when fed a dry ration. They also maintained milk production more satisfactorily on the silage than on the dry ration. It should not be concluded from these results, of course, that pea vine silage is always superior to alfalfa hay. Because of variations in the quality of both silage and hay, a general conclusion could not be drawn until after extended comparisons had been made.

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## THE RELATION OF IODINE AND PEROXIDE NUMBERS TO OXIDIZED FLAVOR OF MILK\*

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Observations on the iodine and peroxide numbers of milk fat have led to conflicting reports as to whether the milk fat itself or the fat globule "membrane" lecithin is the substance oxidized to give this flavor defect. Brown *et al.* (1) found no change in the iodine number of the fat when a copper-induced flavor had developed. Ross (2) found no relationship between the iodine number and the oxidized flavor of ice cream. Several studies (3, 4, 5, 6, 7) of the oxidation of butter and pure milk fat indicate no significant changes in any of the fat constants until the characteristic flavor and the fading of the natural color give unmistakable evidence of an advanced state of deterioration. It has also been reported (8, 9) that the oxidized flavor appears before the end of the induction period of the fat, that is, in the early stages of deterioration. Briggs (3) has shown that peroxides, formed in the process of fat oxidation, do not interfere with or change the iodine value. Therefore a decrease in iodine number coincident with the development of the off-flavor would not necessarily be expected although the milk fat itself were the substance being oxidized to give the flavor. However, Kende (10) and Dahle and Palmer (11) observed a lower iodine number in the fat of spontaneously oxidized samples than in the heated control samples, suggesting that the milk fat itself had been oxidized.

Dahle and Palmer held their samples "until the degree of oxidized flavor did not show any increase," possibly allowing the fat to reach the end of its induction period before the iodine number was determined. Furthermore, their unpublished data show that the milk fat obtained from the milk in all cases was practically colorless even when churned direct from the cow. This suggests that a very pale colored susceptible fat might be associated with the development of the oxidized flavor and with the stability of the fat.

Swanson and Sommer (13) determined the iodine number of the fat and the phospholipid fraction of oxidized flavored milk (copper induced) and found no change in the iodine number of the fat, but a lowering in the iodine number of the phospholipid fraction, indicating that the fat is not affected when an oxidized flavor occurs.

Stebnitz and Sommer (7) found no relation between the carotene content as indicated by the color, and the stability of the fat. This is in agreement with certain observations (11, 12) that the oxidized flavor bears no

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relation to the breed of cow producing the milk. Garrett *et al.* (14) observed that there was a close relation between the per cent fat and the yellow color of the milk, and that a high carotene content is coincidental to and helps preserve a good flavor. On the other hand Beck *et al.* (15) concluded that milk below the breed average in color intensity appeared to be related to oxidized flavor. The beneficial effects obtained by Anderson *et al.* (16) in feeding pure carotene to prevent the development of oxidized flavor might be interpreted to support the findings of Beck *et al.* Although there may be no correlation between the color and stability of milk fat or between the color and oxidized flavor of the milk, there is some evidence that a sub-normal color is associated with the development of oxidized flavor.

#### EXPERIMENTAL

In these experiments milk was obtained from cows known to produce the spontaneous oxidized flavor. Care to avoid possible metal contamination and exposure to light was exercised. Part of the milk was centrifugally separated and the cream standardized to 20 per cent fat by adding some of the original whole milk. The remaining milk and the cream were each divided into three portions, one being unheated, one heated to 80° C. for 10 minutes to inhibit the oxidized flavor, and the other heated to 62° C. for 30 minutes. The heated samples were cooled and stored in the dark at 4.5° C. until examined and analyzed.

Samples of milk and cream known to be resistant to spontaneous flavor were prepared in exactly the same manner except that copper was added to the lots heated at 62° C., after the pasteurizing was completed.

The samples were examined for flavor when fresh and at 24-hour intervals until a pronounced oxidized flavor had developed and again at the time analysis of the fat was made. The iodine and peroxide numbers were determined on the fresh samples and again when a pronounced oxidized flavor had developed and at various intervals during storage. The fat was obtained by churning sufficient milk or cream into butter which was then heated to 60° C. and transferred to a 50 ml. pyrex centrifuge tube, and centrifuged at 3500 r.p.m. for five minutes. The dry, pure, milk fat was removed and the iodine number determined by the Hanus Method and the peroxide number by the method described by Greenbank and Holm (17).

Typical results of the experiments with copper-induced flavor appear in table 1 and indicate no significant change in the iodine number or the peroxide number even when a very strong oxidized flavor had developed. The samples stored for six weeks showed some protein decomposition. A strong oxidized flavor always appeared in the pure milk fat after storage although to the unaided eye there was no apparent fading of the natural color of the fat. Similar findings in regard to the iodine number have been reported before.

TABLE I  
*Relation of iodine and peroxide numbers to copper induced oxidized flavor*

Samples	Fresh		2 days		5 days		3 weeks		6 weeks		
	Flavor	Iodine number	Flavor	Iodine number	Flavor	Iodine number	Flavor	Iodine number	Flavor	Iodine number	
(1) Control milk	-	32.2*	0.0	-	32.6	0.0	-	32.5	0.0	-	
(2) Milk + 2 p.p.m. copper	-	32.2	0.0	++	32.7	0.0	++	32.6	0.0	-	
(3) Control cream	-	32.4	0.0	-	-	-	-	32.5	0.0	-	
(4) Cream + 6 p.p.m. copper	-	32.6	0.0	+	-	-	+	32.5	0.0	+	
								+	32.1	0.0	+
								+	+	+	+

\* Iodine numbers in tables 1 and 3 are higher than they are in table 2. The milk used in table 1 came from another herd as the milk from the station herd failed to produce the spontaneous oxidized flavor.

Since four experiments using spontaneous milk gave the same results, only one experiment is included in table 2. No significant change in either the iodine or peroxide number occurred in samples having an oxidized flavor. The milks showed some protein decomposition after the long storage period at the low temperature.

In holding these samples for such a long period of time a phenomenon occurred which was quite puzzling at the time. The sample heated to 80° C. showed no oxidized flavor but a cooked flavor as has always been the case. Later in the storage period the cooked flavor disappeared and finally after three weeks in storage a strong oxidized flavor occurred. This was contrary to previous findings although previously samples showing the elimination of the oxidized flavor with high temperatures were always discarded after approximately 72 hours. It has been assumed that the high temperature of heating destroyed an enzyme that was responsible for the oxidized flavor.

In view of the work of Gould and Sommer (19), and Josephson and Doan (20) one may attempt to explain this phenomenon on the basis that the high heat produced reducing substances which prevented the development of the oxidized flavor. On long holding at low temperatures the reducing substance became oxidized and the fat or fatty material in turn became oxidized giving rise to the oxidized flavor in this spontaneous milk.

When oxidized flavor occurred in the milk or cream this flavor always carried over in the butter and the pure fat, yet the fat did not change in color. Unfortunately, spontaneous milk having initially a very pale-colored fat was not available for these experiments.

This lack of fading of the natural color indicates, as will be shown later, that the fat had not reached the end of its induction period. This is believed to be a significant factor in explaining the discrepancy between the results of these experiments and the results reported by others showing the relation of iodine number to spontaneous oxidized flavor. While Dahle and Palmer (11) fail to mention the color of the fat in their experiments their unpublished results show that the milk fat obtained from the milk was practically colorless, even direct from the cow. Possibly this pale-colored fat had a very short induction period. The significance of this is emphasized by the experiments to follow.

The fact that the peroxide number of the fat was always zero, even when the fat had a strong oxidized flavor, suggested that the peroxides might have been removed during the isolation of the fat. If such were the case the flavor of the fat could be explained by assuming the flavor substance to be absorbed by the fat and insoluble in water. The following experiment represents many attempts to remove the peroxides by washing the fat with hot water.

Dry, fresh milk fat was obtained from sweet, one-day-old cream that was known to be free from spontaneous oxidized flavor and to be free from any

TABLE 2  
*Relation of iodine and peroxide numbers to spontaneous oxidized flavor*

Treatment of sample	Fresh		2 days		5 days		3 weeks		6 weeks	
	Flavor	Iodine number	Flavor	Iodine number	Flavor	Iodine number	Flavor	Iodine number	Flavor	Iodine number
Milk, unheated	-	28.0	0.0	-	-	-	+ +	28.1	-	27.9
Milk, heated 62° C. 30 min.	-	27.7	0.0	-	-	- + +	-	28.0	-	27.9
Milk, heated 80° C. 10 min.	Cooked	27.8	0.0	Cooked	-	Cooked	27.8	0.0	-	27.8
Cream, unheated	-	28.4	0.0	-	-	-	-	28.0	-	27.8
Cream, heated 62° C. 30 min.	-	28.1	0.0	-	-	+	-	28.2	-	28.4
Cream, heated 80° C. 10 min.	-	28.0	0.0	-	-	-	29.5	0.0	-	28.2

\* This sample developed an oxidized flavor after 3 weeks in storage although it had a cooked flavor prior to that time.

metal contamination. This fresh cream was churned in a glass churn, the butter transferred to a separatory funnel and washed with hot (71° C.) water until the wash water was clear, which required three or four washings with a volume of water equal to the volume of butter each time. The hot fat was centrifuged for five minutes at 3500 r.p.m. The fat obtained was then placed in a carefully cleaned and stoppered flask, and incubated in the dark at 60° C.

The fat was examined at intervals for flavor, peroxide number, iodine number and for any change in color noticeable without the aid of color standards. At certain stages of oxidation, as indicated by the peroxide number, a portion of the fat was transferred to a separatory funnel and washed three times, each time using a volume of clean hot (65° C.) water slightly greater than the volume of the fat. The fat was centrifuged for five minutes at 3500 r.p.m. and the peroxide number and flavor determined.

TABLE 3  
*Flavor and peroxide numbers of washed oxidized fat*

Days in dark at 60° C.	Color	Iodine number	Flavor	Peroxide number	Rewashed fat	
					Peroxide number	Flavor
0	Yellow	33.6	-	0.0		
4	Yellow		±	0.0		
7	Yellow	33.3	+	0.3		
10	Yellow		++	1.8	1.7	++
15	Pale yellow		++	8.0	8.0	++
25	White	31.3	++	46.0	45.5	++

The results in table 3 indicate that neither the peroxides nor the flavored substance are removed by washing the fat with hot water. Therefore the low peroxide number in tables 1 and 2 of fat isolated from milk or cream having a strong oxidized flavor cannot be due to the removal of peroxides during the isolation of the fat. Furthermore, in the autoxidation of pure fresh milk fat the authors have observed the appearance of the oxidized flavor as soon as, or slightly before the appearance of any peroxide number. It, therefore, seems that the determination of the peroxide number is not a sufficiently sensitive method to detect the extremely small concentrations of peroxides which may be produced during the formation of the flavored substance. This undoubtedly explains why Koenig (18) found no suitable correlation between the peroxide number and the flavor of butter.

The observation that the oxidized flavor appears before the end of the induction period has been observed by others (8, 9).

In the autoxidation of pure milk fat the authors noted that the fading of the natural color of the fat occurred with the beginning of the rapid increase in peroxide number, that is, at the end of the induction period. This is indicated in table 3 which also shows that the significant decrease in the

iodine number is more closely associated with fading of the natural color and the end of the induction period than it is with the appearance of the oxidized flavor. This agrees with the report of Briggs (3) who found no significant change in either the peroxide number or the iodine number during the induction period.

Since the oxidized flavor appears early in the induction period, and since the change in iodine number is associated with fading of the natural color and the end of the induction period, it may be possible that a very pale-colored fat would show such a short induction period that the decrease in iodine number would appear approximately at the same time that the oxidized flavor appeared. In such a case, this would explain why Dahle and Palmer (11) using spontaneous milk with a very pale colored fat observed a decrease in iodine number, while the above results on spontaneous milk with a fat having an appreciable color and induction period did not show a decrease in iodine number with the appearance of the oxidized flavor.

#### SUMMARY

That the iodine number of milk fat does not decrease with the early development of the oxidized flavor, agrees with the majority of similar reports. This finding, however, is in conflict with certain investigations on spontaneous milk in which the iodine number was found to decrease.

It was observed that milk fat usually had a peroxide number of zero when freshly isolated, even from milk or cream having a strong oxidized flavor, which was carried over into the pure fat. Evidence is presented indicating that it is not due to removal of the peroxide during the isolation of the fat. It is also shown that the peroxide number and iodine number do not change materially until the color of the fat is affected.

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# A YOLK-BUFFER PABULUM FOR THE PRESERVATION OF BULL SEMEN<sup>1</sup>

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The difficulty of consistently preserving semen has been a serious obstacle in the progress of artificial insemination. In many cases low breeding efficiency has accompanied artificial insemination due to improper or faulty preservation and storage of the semen sample. This is particularly true when it was desired to use a stored sample over a considerable period of time.

In the studies herein reported we attempted to determine certain conditions under which bull semen might be environed to maintain its potency. Our object was threefold: (a) to develop a suitable nutrient medium; (b) to obtain a nutrient solution that would maintain potency for a period of several days, thus eliminating the daily use of the bull; and (c) to develop a procedure which was applicable under practical conditions.

A study of the literature indicates that buffered glucose solutions have been tried by Baker (2), Anderson (1), Molovanov (7), Hyme (5), Walton (10), Winters *et al.* (11), and others. Ivanov (6) and Bernshstein (3) have presented some evidence to indicate that glucose was not the energy metabolite used by spermatozoa. No clear-cut evidence was available to indicate the most favorable storage pH for semen. Hatziolos (4) reported the average pH of 54 samples of bull semen to be 6.89. Shergin's data (9) indicate that the semen of most species is slightly alkaline. He gives a pH value of 6.74 for the bull. Anderson (1) uses a dilutor for ram semen buffered at pH 7.6.

## EXPERIMENTAL

In order to keep these studies on a practical basis it seemed wise to first test naturally-occurring material. Since the egg is the counterpart of spermatozoa it seemed to be a likely starting material. Because of the buffering action of proteins, fresh egg white was used both with and without added glucose. This medium did little if any better than the control sample of raw semen stored under ideal physical conditions.

Next a mixture of fresh egg yolk (hen) and a phosphate buffer was tried. The buffer was made up to a pH of 7.0 from M/15 potassium and

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sodium phosphate solutions and mixed with an equal volume of fresh raw egg yolk. The semen was diluted 4:1 with this mixture. The results were extremely satisfactory. A repetition of the experiment was not so encouraging. A study of the pH of the egg yolks in use showed a wide variation in pH (6.0-6.53). Yolk-buffer mixtures buffered at pH 6.6-6.65 again gave excellent results. These experiments indicated that bull semen could be stored in buffered egg yolk. Egg yolk alone was less effective than buffered egg yolk.

It appeared certain that controlled pH and a buffer system were necessary for the successful storage of semen for long periods. Phosphate buffers of various strengths, M/5, M/10, M/12, and M/15 were tried. Buffering with a stronger solution than M/12 phosphate was found to be detrimental. A series of studies was then made to determine the optimum pH for storage. Semen from ten bulls was stored in yolk-buffer solutions ranging in pH from 6.0-7.5. The optimum pH for storage was found to be 6.75 and the narrow pH range of 6.7-6.8 gave the best preservation of motility. The results are shown in figure 1. If the pH is skewed slightly

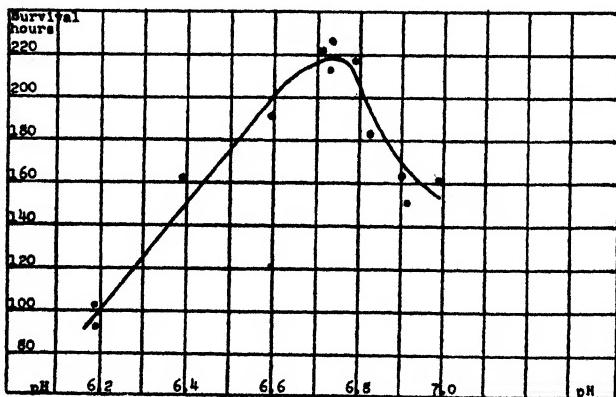


FIG. 1. A representative curve showing the effect of pH of the yolk-buffer upon sperm survival.

to the alkaline side to 6.8-6.9, a low grade (+) motility can be maintained for a longer period than with a pH of 6.75. However, active motility of the order of +++ falls off much more quickly at pH 6.9. Considerable acidity develops during storage. At the end stage the pH is usually near 6.0-6.1. It is possible that the higher pH delays the development of acid in the final stages and thereby increases sperm survival. That this is not entirely the case is indicated by the fact that fresh buffer added from time to time to correct and maintain the proper pH does not prolong survival.

The most favorable ratio of semen: yolk-buffer was next determined. It was found that excellent results were obtained with ratios up to 1:5.

The latter seemed to be the upper practical limit and the best routine results were obtained with a ratio of 1:3 or 4. Optimum storage temperatures indicated maximum storage in the temperature range from 5-10° C. Temperatures outside of this range were definitely inferior.

In connection with these studies numerous other substances and solutions have been tried. A dried pork liver suspension, dried liver extract solution, bovine blood serum and blood plasma, buffered egg yolk fat and materials collected from the testes and accessory glands of the bull were without benefit if not actually harmful to the sperm. Table 1 indicates that some success was obtained with dried egg yolk-buffer and boiled muscle extract.

TABLE 1  
*The comparison of various media with egg yolk-buffer as a pabulum for sperm survival*

Media	Motility and survival time in hours							
	48	72	96	120	150	200	225	300
Buffered glucose	dead							
Buffered lecithin (egg)	+	dead						
Evaporated milk (irrad.)	++	dead						
Chick brain substance	++	-	dead					
Boiled muscle extract	+++	+++	++	dead				
Buffered dry egg yolk	+++	+++	++	dead				
Buffered egg yolk	++++	++++	++++	++++	++++	+++	++	+

These studies thus showed: (a) that egg-yolk furnished the necessary metabolite, or metabolites required by the spermatozoa of the bull, (b) that a buffered solution in a rather narrow restricted pH was necessary, and (c) that the proper combination of these two factors was highly satisfactory for the preservation of bull semen. With these results to guide us, the following procedure was developed and previously published in part (8).

The buffer solution was made up with 0.2 gram of  $\text{KH}_2\text{PO}_4$  and 2.0 grams of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  per 100 cc. of distilled and sterilized  $\text{H}_2\text{O}$ . This buffer can be kept in the refrigerator for short periods but works best when made up fresh. Equal amounts of the buffer and fresh egg yolk are then mixed. The proportion of buffer in this preparation is designed to yield a mixture whose final pH is 6.75. It should be checked before use and if need be adjusted. In 90 per cent of the cases the pH will be 6.75. Adjustment of pH is made with sterile solutions of either the monobasic potassium, or dibasic sodium salt as required. Yolk-buffer

mixtures with a pH lying between 6.7-6.8 will be found to be satisfactory. The yolk-buffer preparation is now ready for use.

Freshly collected semen (the second ejaculate is often preferable where the bull is not used regularly and often enough) was then combined with the yolk-buffer in the proportion of 1:3 or 1:4. The yolk-buffer semen sample was then stored in stoppered containers kept in the refrigerator at a temperature of 10° C. No undue care has been found necessary in the cooling or warming of the sample. One can go from room temperature to 10° C. and back to room temperature without serious damage.

Semen thus preserved maintains its original activity with remarkable consistency. A high grade, very active semen will maintain a high degree of activity for 100 hours and more. Semen from the bulls used in these tests maintained excellent activity up to 150 hours. Thereafter there was a gradual loss of motility during the next 100 hours. It was not at all uncommon for some activity to last beyond 300 hours.

Semen suspended in yolk-buffer has been successfully shipped from British Columbia to the middle west and from coast to coast.

In much of this experimental work we have relied upon the microscope and sperm motility for the detection of semen quality. It appears that, within certain limits, a yolk-buffer-semen sample which shows vigorous activity under the microscope will usually be found to be potent. Table 2 indicates that yolk-buffered semen is potent for a considerable time.

TABLE 2  
*Summary of breeding records with yolk buffered semen on cows bred for sixty-three or more days*

Y.B. semen age (hrs.)	No. of cows bred	Total services	No. diagnosed pregnant	Total* No. apparently pregnant	No. poor breeders**
1-12	28	33	9	20	14
12-24	11	12	1	5	3
24-36	58	68	17	35	21
36-48	2	2	1	2	1
48-60	14	15	5	5	6
60-72	2	2	0	1	1
72-84	2	4	1	1	2
84-96	0	0	0	0	
96-108	8	8	1	2	4
124	1	1	1	1	0
151	1	1	1	1	0
180	1	1	1	1	0
196	1	1	0	0	1***

\* This figure includes the cows diagnosed pregnant and those apparently pregnant but undiagnosed. At least 63 days had elapsed since breeding and without reoccurrence of oestrus.

\*\* Cows with a history of repeated breeding.

\*\*\* This cow had been previously bred 3 times.

These data were compiled from two breeding rings and the University herd. At the moment it has not been possible to have pregnancy diag-

noses on all cows inseminated with yolk-buffered semen. Two problems have come to our attention in this connection. One is the fact that there has been a "repeater" list (cows that require more than one service to settle) in this group of breeding cows. In these experiments the repeaters averaged slightly more than 40 per cent of the animals under observation. Secondly, a certain group of cows would apparently settle after service and skip several heat periods following insemination but later they show estrus and rebreed. Whether or not pregnancy occurred in these cows could not be determined. As far as we can ascertain these two problems are present in the herd irrespective of natural or artificial service used in the insemination. The yolk-buffer did not produce or prevent this condition. In view of the excellent results obtained in these experiments, it appears that the egg yolk constituents were completely metabolized or eliminated without harm to the cow.

One more point should be made clear. There is a great variation in the quality of bull semen. The yolk-buffer solution does not improve a poor sample of semen. We have noticed a distinct difference in the length of time semen can be preserved from bulls of different potency ratings. The bull that produces an excellent semen with high initial activity can be successfully stored for longer periods than the semen from a bull that produces a less vigorous semen sample.

#### SUMMARY AND CONCLUSIONS

A method for the preservation of bull semen has been described. This method makes use of egg-yolk buffered at pH 6.75 as the pabulum for spermatozoa. By this means the fertilizing capacity of vigorous spermatozoa has been regularly maintained for periods in excess of 100 hours, if stored at 10° C. This method has been successfully used under practical conditions.

Under these environmental conditions bull semen can be successfully stored to give consistent preservation of its fertilizing capacity under practical conditions. Yolk-buffer maintains potency equivalent to the original semen for a variable period depending upon the quality of the fresh semen sample. Cows have been successfully bred with yolk-buffered semen stored for 150 to 180 hours.

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# THE COAGULATION TEMPERATURE OF MILK AS AFFECTED BY pH, SALTS, EVAPORATION AND PREVIOUS HEAT TREATMENT

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The stability of milk under various conditions has been studied from both a theoretical and practical standpoint. The milk has been subjected to temperatures ranging from room temperature to 150° C., and to the influence of salts and acids that are foreign as well as those normal to milk. Although valuable information has been added to our knowledge of milk, yet many theoretical and practical questions have not been answered which are significantly related to the stability of milk during pasteurization, boiling, preparation of various food-stuffs involving milk, and the manufacture of condensed and evaporated milks. The purpose of the work presented herein is to show the relationship between the pH and the coagulation temperature of milk ranging from about room temperature to the coagulation temperature of stable milk, and to show the effect of added salts, evaporation and previous heat treatment in displacing the "coagulation curve."

## HISTORICAL

It has been known for a long time that fresh milk would coagulate at elevated temperatures. Hammarsten (10) in 1874 reported that the coagulation temperature varied from 130 to 150° C. Cazeneuve and Haddon (5) attributed the curdling of milk at 130° C. to the formation of acids, mainly formic, from lactose oxidation. Bardach (1) failed to confirm this conclusion. Milroy (20) showed that heating slightly increased the acidity of milk. More recently Whittier and Benton (43, 44, 45) concluded that the rate of acid production during heating at 90 to 120° C. was a direct function of the time and temperature of heating, and lactose concentration. Their results indicate that acid production may become an important factor with long holding periods at elevated temperatures. Holm, Deysher and Evans (11) concluded that the relationship between time and temperature of coagulation was of a logarithmic nature.

The early workers, such as Stokes (33), Thörner (37), Rideal (25), Richmond and Harrison (24) and Kastle and Roberts (14), showed that the acidity-curdling temperature relationship varied considerably between milks. Recently Benton (2) reported that wide variations existed even in the milk from different quarters of the same udder. The aims of the present paper are most closely indicated by the work of Tapernoux and Katrandjieff (35) in which the change in titratable acidity, pH and approximate curdling temperature was determined for a sample of milk held at 14° C.

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The early workers observed that not only an increase in acidity but also the addition of salts would lower the coagulation temperature of milk. Ringer (26) in 1890 was one of the first to report such findings. Conradi (6) found that the addition of 0.2 to 0.6 per cent calcium chloride would coagulate milk at 45 to 65° C., while larger or smaller amounts would not coagulate the milk. Loevenhart (19) likewise found an optimum concentration of  $\text{CoCl}_2$ ,  $\text{CaCl}_2$ ,  $\text{MnCl}_2$ , and  $\text{NiCl}_2$  for the coagulation of milk at 40 to 60° C.

Within recent years the problem of the heat stability of milk has received considerable attention due to the development of the evaporated milk industry. Sommer and Hart (30) in 1919 studied the coagulation of milk from individual cows at 136° C. and found that some samples coagulated in 90 seconds while others failed to coagulate in 20 minutes. No relationship was found between the coagulation and the titratable acidity or pH of the fresh milk samples. The differences in coagulation were found to be due mainly to the relative amounts of calcium and magnesium as compared to the citrates and phosphates present in the milks. An excess of either of these two groups caused decreased stability; for maximum stability they must be present in the proper ratio or balance. Rogers, Deysher and Evans (27) in 1921 found no correlation between the coagulation of the raw mixed herd milk and the same milk after evaporation. They were also unable to find by means of chemical analysis any relation between the acid-base ratio in the raw milk and the coagulation temperature of the evaporated product. However, Sommer and Hart (31) in 1922 showed conclusively that evaporated milks could be stabilized by small (within limits of analytical experimental error) additions of citrates or phosphates and the same milks destabilized by calcium.

Various investigators have confirmed the conclusion that salts are an important factor in the heat stability of milk and milk products; namely, Benton and Albery (3), Whitaker (42), Holm, Webb and Deysher (12), Webb and Holm (40), Welch and Doan (41), and Tracy and Ruehe (38). The results of Webb and Holm, and Holm, Webb and Deysher have been summarized (18) to show three types of milk. All skim milks of normal concentration are reported to be stabilized by phosphate and destabilized by calcium. When the skim milks were concentrated to half their original weights, they obtained type I, which was destabilized by calcium and first stabilized, then destabilized by larger additions of phosphate; type II, destabilized by both calcium and phosphate; and type III, destabilized by phosphate and first stabilized, then destabilized by additions of calcium. Welch and Doan (41) found that milk from cows with subclinical mastitis reacted like type III milk.

Sommer and Hart (32) found cases where the milk could be improved by small additions of acids. These results have been confirmed by sev-

eral other investigators. Benton and Albery (3) found that within the pH range of 6.58 to 6.65 the salt balance changes had a marked effect, while outside this range, changes in pH were the predominant factor. They concluded each sample of milk had its own optimum of pH and salt balance. A similar conclusion was reached by Webb (39) and Ramsdall, Johnson and Evans (23).

The probable role of rennet-producing organisms in the heat coagulation of milk was studied by Frazier (9). The effect of renin, acid and calcium appeared to be additive.

Previous heat treatment has been shown to be an important factor in the heat stability of milk. Conradi (6) in 1901 found that if the milks were first heated to above 60° C., the coagulation on addition of calcium chloride occurred at temperatures of 8 to 12° C. lower than in the case of unheated controls. Webb and Holm (40) found that milks with a solids-not-fat content of around 13 per cent were not affected by forewarming, but milks of lower solids were destabilized while milks of higher concentrations were stabilized. Various other workers have reported beneficial effects upon the concentrated product from forewarming (27, 7, 17, 16, 32). The results show the optimum forewarming temperature to be near 100° C. when heated for ten minutes. The relationship between temperature, time, method of forewarming and concentration has not been satisfactorily determined.

The effects of forewarming may be attributed to a number of factors. Söldner (28) in 1888 showed that boiling caused a precipitation of a portion of the calcium phosphate from milk. This has been confirmed by many other investigators. Palmer (22) concluded it was the colloidal  $\text{CaHPO}_4$  that was affected by heating, however, beneficial effects of forewarming suggest the precipitation of soluble calcium salts. Sommer and Hart (32) found larger amounts of soluble calcium in samples of milk heated at 180° F. than when heated at 210° F. Sommer (29) showed that part of the beneficial effect was due to the precipitation of albumin. Svedberg, Carpenter and Carpenter (34) found that the molecular weight of pure casein was doubled by heating to 40° C. On the other hand, Nichols, *et al.* (21) concluded that the particle-size distribution of the calcium caseinate in skim milk was not affected by preheating at 65 to 95° C. Howat and Wright (13) found that heating calcium caseinate at 120° C. for 1 to 5 hours liberated free phosphorus and nitrogen, lowered the base-binding capacity and liberated calcium with the result that the product was less heat stable.

#### EXPERIMENTAL

One of the difficulties in studying the heat stability of milk is the lack of a satisfactory method for measuring the coagulation point of the milk. The results shown in figure 1 were obtained by forcing the milk by means

of air pressure through small glass tubing extended through a water bath in which the temperature was gradually increased, and allowing the heated milk to drip at a constant rate onto a black plate glass. The occurrence of coagulation was easily detected by observing the milk as it flowed over the black glass, and at the instant of coagulation the temperature of the water bath was taken as the coagulation temperature of the milk. An adaptation of this plan for measuring coagulation at elevated temperatures involved many difficulties and was abandoned.

The method finally deemed most satisfactory for high temperatures was a modification of the one used by Sommer and Hart (30). This consisted of sealing the milk in glass tubes made from 8 mm. pyrex tubing, placing the tubes of milk in a rack which was then immersed in a thermostatically controlled oil bath. The rack was clipped onto a shaft which was rocked back and forth by mechanical means so that the milk flowed from one end of the tubes to the other, with a pause at each position to allow complete flow of milk. Therefore, uniform agitation was obtained and at the same time the coagulation time could be detected by noting the flow of the milk. In order to decrease as much as possible the factor of acid production from lactose oxidation, a time interval just ample to allow the sample to reach the oil bath temperature was chosen. Thermocouple measurements showed that the milk came up to the temperature of the bath in 85 to 110 seconds; hence, the coagulation point or temperature was fixed as that temperature at which the milk would coagulate in just

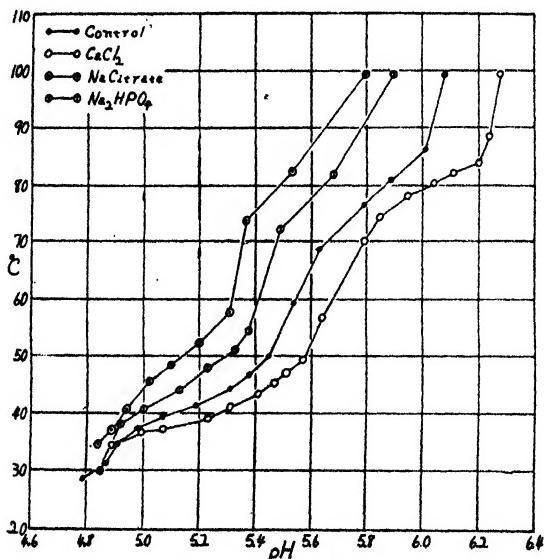


FIG. 1. The effect of salts and pH on the coagulation temperature of milk at temperatures below 100° C. 2 ml. M/4 salt added per 100 ml. skim milk.

two minutes from the time the sample was placed in the oil bath. Since numerous tests would be necessary to determine the temperature which would cause coagulation in exactly two minutes, the coagulation time was determined at temperatures above and below this time limit. These results were plotted, and the coagulation temperature for a two minute heating period was obtained by interpolation. For each sample a series of sub-samples was prepared ranging from about pH 5.0 to 6.8. The temperature required to produce coagulation in two minutes was determined for each sub-sample and plotted against the pH. The curve plotted in this manner is designated as the coagulation curve of the sample.

The acidification of sub-samples was accomplished by fermentation in the case of figure 1, and by acid additions in all subsequent experiments. This was done by adding diluted lactic acid in a very fine stream with the milk in vigorous agitation. To study the effect of the dilution involved, acidification of some samples was accomplished by agitating the milk in contact with air containing controlled amounts of HCl gas, with and without dilution.

Due to the amount of work involved, it was necessary to make up the samples one day, store them in the ice box and run the coagulation points and pH the following day. The pH was determined with the quinhydrone electrode, the temperature being maintained at 25° C. by means of a thermostatically controlled water bath.

A comparison of the methods of acidification (Curves G, H, I, Fig. 2) showed no detectable effect on the coagulation curve below pH 6.4. The control sample in figure 1, acidified by fermentation, shows a slightly different curve. Without further study it is not known to what extent the difference is due to other effects of fermentation, *e.g.*, citrate destruction, or to the method of heating.

Added salts displaced the coagulation curves as shown in figures 2 and 3. This displacement was into the more stable region by  $\text{Na}_2\text{HPO}_4$ ,  $\text{Na}_3\text{C}_6\text{H}_7\text{O}_5$  and  $\text{K}_2\text{CO}_3$  and into the less stable region by  $\text{CaCl}_2$  and  $\text{Ca}(\text{C}_2\text{H}_5\text{O}_2)_2$ , except at a pH above 6.5. The addition of phosphate, citrate or carbonate to the fresh skim milk (pH above 6.5) lowered the stability of the skim milks. The amount of calcium added also decreased the heat stability, but a smaller addition of calcium would have increased the heat stability of these skim milks as is revealed by results recorded in table 1. Since these milks at their initial pH were deficient in calcium for maximum heat stability, the first increases in acidity had an effect analogous to that of adding small amounts of calcium salts.

The results presented in figure 4 show that the addition of salts at a rate which produces maximum effects in the fresh milk, as indicated in table 1, has very little effect at lower pH. A portion of this sample condensed 2:1 illustrates the form of coagulation curve given by condensed

milk. The condensed portion reacted still less, but similar to that of the original, to the addition of phosphate, calcium and acid.

Figure 5 shows the effect of larger additions of salts on the coagulation curve of the skim milk sample before and after condensing. Adding various amounts of calcium and phosphate, from minute to large quantities, showed that the condensed portion was destabilized by both salts.

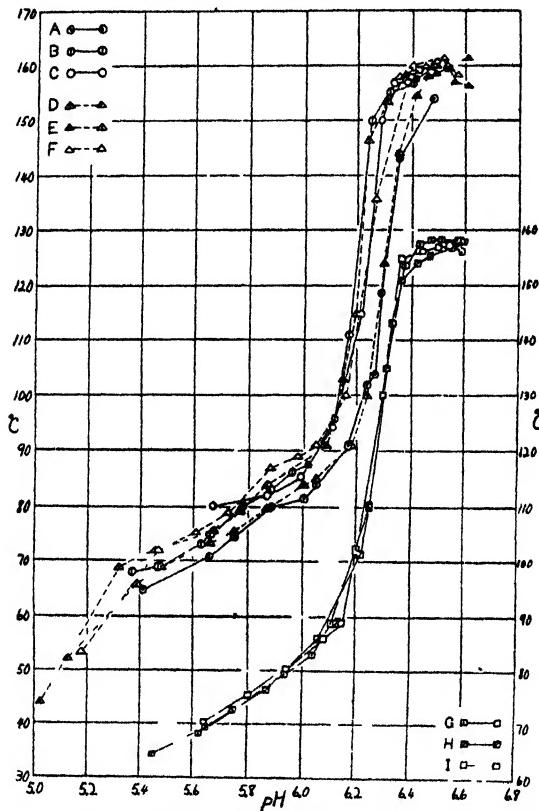


FIG. 2. The coagulation temperature of skim milk as affected by pH, salts and method of acidification.

Added to 100 ml. of skim milk (before acidified with HCl vapors) - 2 ml. of:

A—water	I)—water
B—M/4 Na <sub>2</sub> HPO <sub>4</sub>	E—M/4 Na <sub>2</sub> PO <sub>4</sub>
C—M/4 K <sub>2</sub> CO <sub>3</sub>	F—M/4 K <sub>2</sub> CO <sub>3</sub>

Added to 100 ml. of skim milk:

- G—5 ml. water; then acidified with HCl vapors.
- H—5 ml. dilution; acidified with lactic acid solution.
- I—no dilution; acidified with HCl vapors.

Temperature scale on the right relates to G, H, & I.  
Temperature scale on the left relates to A, B, C, D, E, & F.

On the other hand, the curves show that the sample was increased in heat stability by adding acid.

The similarity of the effects of preheating and pH, and of evaporation and pH on the coagulation temperature of skim milk is illustrated in figure 6. Curve A gives the coagulation curve of the original skim milk and curve F shows how it was displaced merely by preheating. These are

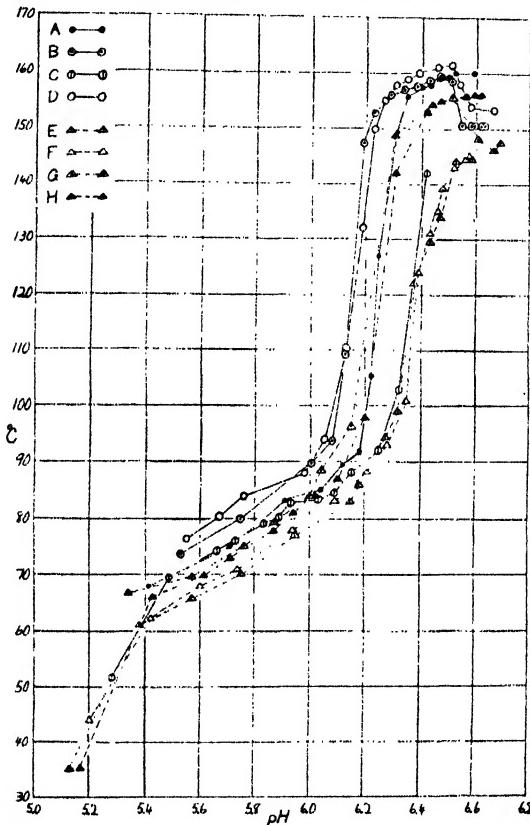


FIG. 3. The coagulation temperature of skim milk as affected by pH and salts.  
Added to 100 ml. of skim milk (before acidified with HCl vapors) - 2 ml. of:

A—water

B—M/4  $\text{Na}_2\text{HPO}_4$

C—M/4  $\text{CaCl}_2$

D—M/4  $\text{Na}_4\text{P}_2\text{O}_7$

Added to 100 ml. skim milk (before acidified with 3 ml. lactic acid solution) - 2 ml. of:

E—M/4  $\text{CaCl}_2$   
 F—M/4  $\text{Ca}(\text{C}_2\text{H}_5\text{O}_2)_2$   
 G—M/4  $\text{Na}_2\text{PO}_4$   
 H—water

$\left. \right\}$  pH above 6.4 by adding NaOH

compared to the displacement by condensing the preheated sample (curve M) and by condensing the unpreheated sample (curve P). Heating at 90-98° C. for 30 minutes made the skim milk of normal solids content less stable to heat at pH values above 6.5 and below 6.1. The concentrated portions showed the same trend in that the preheated sample was less stable than the sample not preheated at pH values above 6.15 and below 5.75.

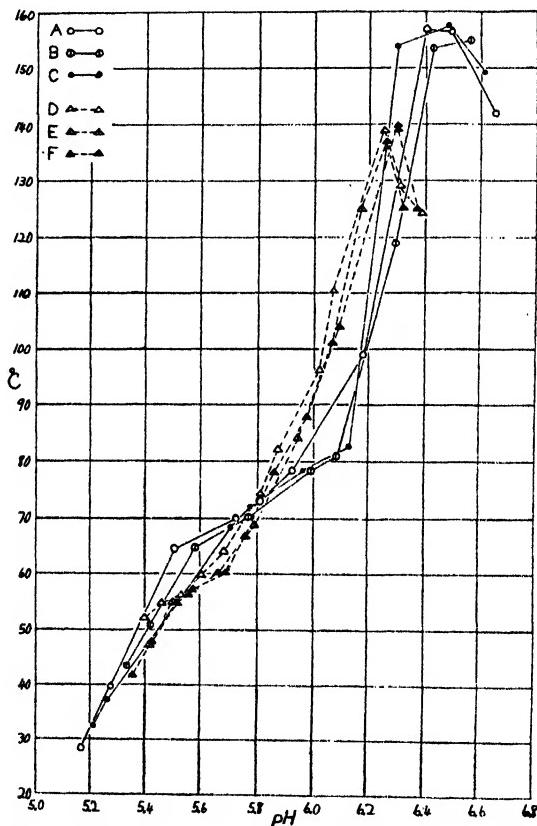


FIG. 4. The coagulation temperature of skim milk as affected by pH, salts, and evaporation.

Skim milk—to 100 ml. added (before acidifying with 4 ml. lactic acid) 0.8 ml. of:

- A—M/4  $\text{Na}_2\text{HPO}_4$
- B—M/4  $\text{Ca}(\text{C}_2\text{H}_5\text{O}_2)_2$
- C—water

Skim milk preheated at 98° C. for 5 minutes and evaporated 2:1 at 59° C., 100 ml. portions treated as above:

- D—M/4  $\text{Na}_2\text{HPO}_4$
- E—M/4  $\text{Ca}(\text{C}_2\text{H}_5\text{O}_2)_2$
- F—water

TABLE I  
The effect of calcium and phosphate on the coagulation time of skim milk

25 ml. milk		ml. water	0.0	0.6	0.35	0.5	0.4	0.3	0.2	0.1	0.0
plus		ml. M/4 salt	0.0	0.0	0.05	0.1	0.2	0.3	0.4	0.5	0.6
Coagulation at 136° C. in minutes											
Sample A											
Calcium acetate	2: 40	3: 05	4.25	21	47	1: 50	4.5	45	2: 00	2: 00	0: 35
Disodium phosphate	2: 40	3: 05		2: 15			1: 55		1: 55		2: 00
Sample B											
Calcium acetate	3: 05	3: 05	4: 20	13	34	29	28	27	27	27	0: 45
Disodium phosphate	2: 05	3: 05		2: 30	2: 25	2: 20	2: 15	2: 15	2: 15	2: 15	2: 30

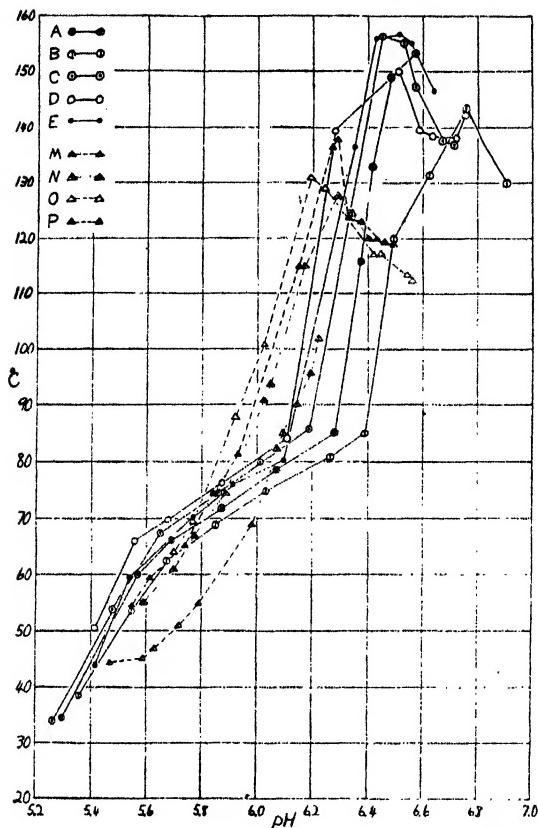


FIG. 5. The coagulation temperature of skim milk as affected by pH, salts and evaporation.

Skim milk—8.6% T.S.—to 100 ml. added (before acidifying with 2.8 ml. lactic acid solution) 1.2 ml. of:

- A—M/4  $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$
- B—M/2 " —pH above 6.5 by adding NaOH
- C—M/4  $\text{Na}_2\text{HPO}_4$
- D—M/2 "
- E—water

Skim milk—16.5% T.S.—100 ml. portions treated as above:

- M—M/1  $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$
- N—M/4  $\text{Na}_2\text{HPO}_4$
- O—M/1 "
- P—water

The curves of figure 7 show that calcium and phosphate displaced the coagulation curve of the samples represented in figure 6 in the usual way; that is, after the milks have reached their maximum stability further increase in the H-ion concentration caused the phosphate to displace the

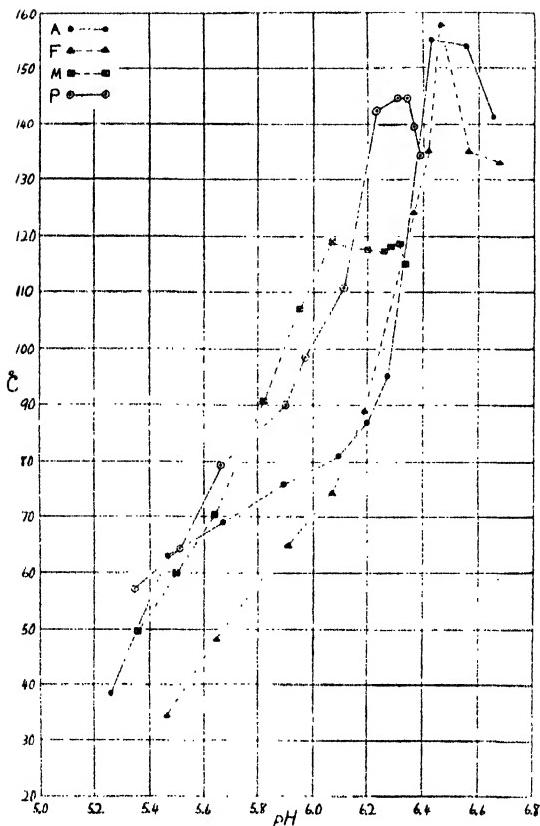


FIG. 6. The coagulation temperature of skim milk as affected by pH, preheating and evaporation.

A—Skim milk 8.7% T.S.; not preheated; diluted 4.4 ml. per 100 ml.

F—Skim milk 8.7% T.S.; preheated at 90-98° C. for 30 minutes; diluted 4.4 ml. per 100 ml.

M—Skim milk 20.1% T.S.; preheated at 90-98° C. for 30 minutes before evaporation; diluted 8 ml. per 100 ml.

P—Skim milk 19.6% T.S.; forewarmed only to 60° C. before evaporation; diluted 8 ml. per 100 ml.

the coagulation curve into the more stable region while calcium displaced the curve into the less stable region. A possible explanation of this is indicated by the results in table 2. It is shown that as the temperature of the milk is increased from 20 to 60° C., the pH of the milk is lowered. The presence of added calcium in the milk caused the pH of the milk to be decreased more rapidly while added phosphate caused the decrease to be less rapid than in the normal milk containing no added salts.

An increase in the acidity of milk with increased temperature was pointed out by Duncombe (8) in 1924. Zoller (46) had shown in 1920 that solutions of milk salts attained a higher pH after they had been heated in the presence of additional citrates. The changes in pH of various buffer solutions with temperature have been studied by many workers. As an example of the results, Kolthoff and Tekelenburg (15) reported that a 0.05 M potassium acid phthalate solution increased in pH from 3.94 at 18° C. to 4.05 at 60° C. and a 0.1 M tri-sodium citrate solution decreased in pH from 9.18 at 18° C. to 8.82 at 60° C. The significance to the heat stability of milk of various physico-chemical equilibria at elevated temperatures is further emphasized by the following formula for the iso-electric point of a simple monobasic, monoacidic ampholyte (36) :-

$$(H^+) = \sqrt{\frac{k_a k_w}{k_b}}$$

TABLE 2

*The effect of calcium and phosphate on the change in pH of milk with increased temperatures*

Temperature	M/20 potassium acid phthalate		100 ml. skim milk plus 2 ml. of					
			water		M/4 CaAc		M/4 Na <sub>2</sub> HPO <sub>4</sub>	
	pH	± pH at 20°	pH	± pH at 20°	pH	± pH at 20°	pH	± pH at 20°
20	3.93	.....	6.64	.....	6.51	.....	6.75	.....
30	3.97	0.04	6.55	0.09	6.37	0.14	6.65	0.10
40	3.99	0.06	6.45	0.19	6.27	0.24	6.60	0.15
50	4.02	0.09	6.34	0.30	6.14	0.37	6.51	0.24
60	4.07	0.14	6.23	0.41	6.05	0.46	6.35	0.40

Note. Ecalomel N/10 from International Critical Tables 1: 81. 1926.  
 $E_k = 0.7175 - 0.00074t$  (4).

The value of  $k_w$ , the dissociation constant for water, increases markedly with temperature. The effect of temperature on  $k_a$  and  $k_b$  for milk proteins is not known. It is conceivable that coagulation at elevated temperatures may be due to shifts in these constants such that the iso-electric point is approached.

The effect of pH on the coagulation temperature of the various original skim milks represented in the previous curves is summarized in figure 8. Three of the skim milks were initially unstable. Merely by decreasing the pH, they were stabilized to a coagulation temperature above 152° C. Between pH 6.6 and 6.4 the coagulation temperature showed very little change with pH. Decreasing the pH from 6.4 to 6.2 rapidly decreased the coagulation temperature from near 155° C. to about 90° C. Then decreasing the pH from 6.2 to about 5.4 gave a very gradual decrease in coagulation temperature from about 90° C. to near 65° C. Points below pH 5.4 were

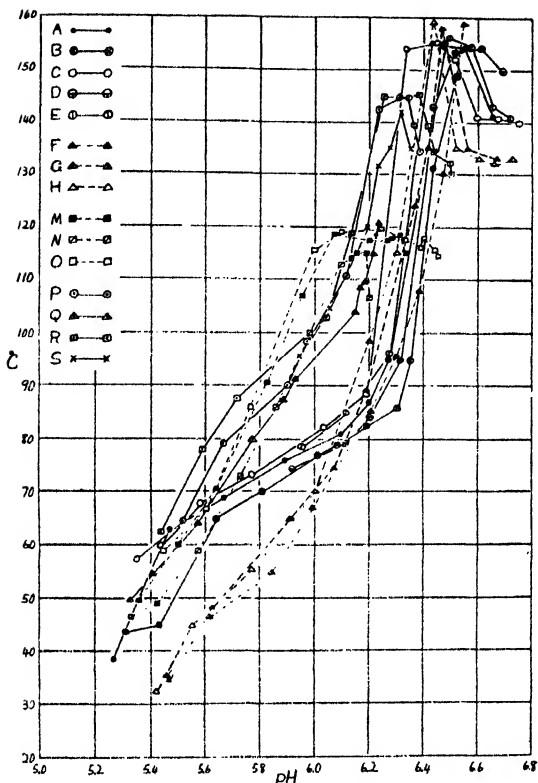


FIG. 7. The coagulation temperature of skim milk and of the evaporated skim milk as affected by pH, salts and heat treatment.

Skim milk—8.7% T.S.; not heated; to 100 ml. added (total dilution 4.4 ml. due to salt sol'n, lactic acid and water):

- A—water
- B—2 ml. M/4 calcium acetate
- C—2 ml. M/4 di-sodium phosphate
- D—0.8 ml. M/4 calcium acetate
- E—0.8 ml. M/4 di-sodium phosphate

Skim milk—8.7% T.S.; heated for 30 min. at 90–98°C.; to 100 ml. added (total dilution 4.4 ml. etc.): F—water

- G—1.6 ml. M/4 calcium acetate
- H—1.6 ml. M/4 di-sodium phosphate

Skim milk—20.1% T.S.; preheated 30 min. at 90–98°C.; to 100 ml. added (total dilution 8 ml. etc.): M—water

- N—4 ml. M/4 calcium acetate
- O—4 ml. M/4 di-sodium phosphate

Skim milk—19.6% T.S.; forewarmed to 60°C.; to 100 ml. added (total dilution 8 ml. etc.): P—water

- Q—4 ml. M/4 calcium acetate
- R—4 ml. M/4 di-sodium phosphate
- S—1.2 ml. M/4 calcium acetate

not very definitely determined, however, the results indicate that decreasing the pH from 5.4 to about 5.2, decreased the coagulation temperature from about 65° C. to near 35° C. It also appears that decreasing the pH from 5.2 to 4.7, the iso-electric point of casein, would give another region of gradual decrease in coagulation temperature.

#### SUMMARY AND CONCLUSIONS

1. Sealing the milk in small glass tubes and subjecting them to a rocking motion in an oil bath was found to be a convenient method for studying the heat coagulation of milk. The milk came up to the temperature of the oil bath in less than two minutes.
2. The relationship between pH and coagulation temperature of skim milk formed a characteristic coagulation curve. The milks were very sensitive to pH changes within two pH ranges; from approximately pH

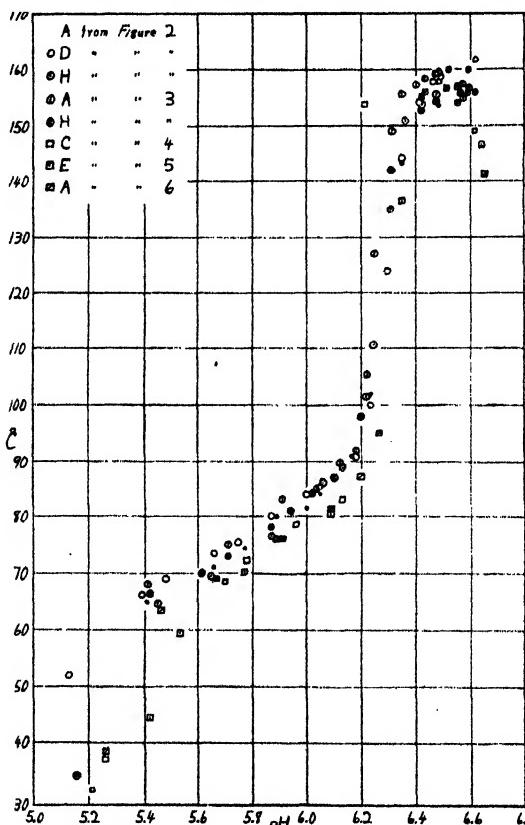


FIG. 8. The effect of pH on the coagulation temperature of skim milks represented in figures 2 to 7.

6.4 to 6.2 and to a lesser extent between pH 5.4 and 5.2. The maximum coagulation temperature of all the milks studied was above 152° C. The coagulation temperature of milks initially less stable than this was increased in heat stability by adding acid.

3. The presence of added salts displaced the coagulation curve; at pH values below 6.4, phosphate displaced the curve into the more stable region whereas calcium had the opposite effect. The effect at pH values above 6.4 depended upon the milk in question. The milks were initially destabilized by adding phosphate and the subsequent addition of acid restabilized the samples. The addition of small amounts of calcium stabilized the milks but larger additions destabilized the milks.

4. Concentrating and preheating displaced the coagulation curve of the milks in the same general manner, but the effect of concentrating was much greater than the effect of heat treatment alone. The presence of added salts displaced the coagulation curve for concentrated and preheated milks in a manner similar to that of the original milk.

5. The pH of the milk was decreased at increased temperatures; the presence of added calcium increased the effect of temperature changes while phosphate had the opposite effect. A more detailed study of various physico-chemical equilibria in milk at elevated temperatures would therefore appear to be a definite contribution to the problem of the heat stability of milk.

6. These conclusions are based on tests involving definite conditions, such as heating and holding so that coagulation occurred in two minutes, and do not necessarily represent the behavior of milk under other conditions.

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## THE KEEPING QUALITY OF BUTTER

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A factor in butter merchandising which is coming in for increasingly greater emphasis is the keeping quality of the product. The ability of any given lot of butter to retain its desirable flavor and odor characteristics through regular trade channels and into the hands of the ultimate consumer is a vital consideration in the manufacture and sale of high quality butter. The present discussion deals with the problem of butter keeping quality from the standpoint of the butter manufacturer and the butter merchandiser. It involves keeping quality examinations on 22,060 samples of creamery butter, each sample representing a regular commercial churning. The writer personally visited all creameries concerned at frequent intervals. The object of this work has been to determine by practical methods, the causes and elimination of defective keeping quality under commercial conditions.

### HISTORICAL

An extensive review of the literature pertaining to butter keeping quality was presented by Herried and associates (1) and later by Jacobsen (7). In the current study it was desirable to confine observations to butter defects which developed in fresh butter at ordinary refrigeration temperatures ( $30^{\circ}$  F.- $45^{\circ}$  F.) in short periods of time. For this reason only those publications directly concerning defects such as putrid or cheesy, and rancid flavor developments are considered.

There is a considerable lack of agreement among numerous investigators as to the precise causes of off flavor developments. Also there are some differences of opinion with regard to the most satisfactory tests for evaluating the keeping quality of butter in the laboratory.

Hunziker (4) describes limburger flavor as a flavor development in butter which is not common, but which occurs spasmodically and is not infrequently epidemic. This author also emphasizes the importance of water purity as an important consideration in producing butter which keeps properly.

Hood (3) reproduced surface taint in butter by artificial inoculation of butter with water bacteria and indicated water contamination as a major cause of this type of difficulty with butter keeping quality.

Herried (1) and associates found that raw cream in a majority of cases contained organisms capable of producing cheese-like flavors in unsalted butter. Pure cultures isolated from this source and mixed cultures

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of bacteria from raw cream and from creamery water were inoculated into sterile cream and were found to produce cheesy flavors in the resulting butter. Creamery water was sometimes found to contain organisms capable of producing cheesy flavors in unsalted butter when inoculated into sterile cream.

In a study of seventeen samples of rancid butter, Hussong and Associates (6) found *Pseudomorpha fragi* present in 16 of the samples. This organism was found in numerous other dairy products. It was found to be capable of rapid growth at 10° C. (50° F.) to 30° C. (86° F.) but was killed by incubation for several days at 37° C. The presence of this organism, found to be easily killed by heat, in pasteurized products was taken as an indication of contamination subsequent to heat treatment.

Jacobsen (8) made a study of bacterial content of butter before and after storage and observed the keeping quality of salted and unsalted butter before and after storage for 90 days at -25° C. (-13° F.). Decreases in numbers of bacteria during storage were observed in both unsalted and salted butter but decreases in counts were greater in the salted butter. No defective keeping quality was found in the salted butter after storage but some of the unsalted made from the same churning developed flavor defects upon being submitted to a keeping quality test involving holding samples in sterile jars at room temperature for 8 days.

Nelson (10) observed the changes in numbers of bacteria in butter held 7 days at 21° C. by the microscopic method and by the plate method. This investigator found that large numbers of gram negative rods were commonly associated with poor keeping quality. No correlation between keeping quality and plate counts of butter was observed.

Olson and Hammer (12) studied the keeping quality of butter in relation to churn contamination and found rancidity to be the most common defect developing in butter as a result of contaminated churning. Samples studied were held at 32° F. and 45° F. The flavor deterioration was found to occur at 45° F. more rapidly than at 32° F.

Olson (11) made studies on the keeping quality and bacterial counts of butter made using artificially contaminated water. A Sietz filter was employed for removal of bacteria from water before washing butter. No appreciable difference in numbers of bacteria in butter from filtered or unfiltered water was noted in the butter as judged by the plate count but significant differences in keeping quality of the butter were noted in favor of the filtered water.

Using an especially adapted technique, Long and Hammer (9) investigated the effect of moisture dispersion in butter on the growth of bacteria in butter. They observed the keeping quality of butter made from cream inoculated with organisms isolated from defective butter. The degree of division of moisture droplets in butter (working) was found to markedly

influence the rate of deterioration. It was found that almost without exception, thoroughly worked butter had by far the best keeping quality, underworked butter deteriorated very rapidly while moderately worked butter showed intermediate keeping quality values. This work doubtless explains the lack of correlation in some earlier observations on butter keeping quality and causes of butter deterioration.

Hammer (2) has observed cases of roquefort cheese-like flavor development in butter which was commonly caused by mold growth even though molds were not evident until microscopic examinations were made. This author (2) also made many keeping quality observations on samples containing various butter spoilage organisms and indicated that unfavorable keeping quality at 40° F. to 45° F. was duplicated at 70° F.

It is generally agreed that defects which develop in butter held at ordinary refrigeration temperatures (30° F. to 50° F.) can be duplicated in shorter periods of time at higher temperatures. This fact affords a means of determining the relative keeping quality possessed by a lot of butter by simply holding a small sample for seven days to two weeks at a temperature between 60 and 70° F.

Nelson (10) employed a holding test for 7 days at 21° C. to evaluate the keeping quality of a number of churning of commercial butter and exhibition butter. Jacobsen (7) describes a keeping quality test at room temperature for 7 to 10 days using sterile glass jars for holding sample as a valuable means of evaluating the keeping properties of unsalted and salted butter.

Parsons (13) employed a keeping quality test using a temperature of 60° F. for 14 days on regular parchment wrapped prints to examine commercial churning over a period of five years and submitted thousands of churning to this test.

#### KEEPING QUALITY TESTS

The present study employed a keeping quality test wherein a one-fourth pound sample of each churning was wrapped in commercial parchment wraps and held seven days at 68 to 70° F. in a thermostatically controlled cabinet. All samples were examined for flavor and odor defects at the end of this period. Samples were reported as satisfactory, fair or unsatisfactory. When off flavors developed they were classified as slightly or definitely cheesy, putrid, rancid, etc., according to the type and extent of flavor development. A total of 22,060 churning of both salted butter, containing 1.8 to 2.3 per cent salt, and unsalted butter from more than 100 plants located in 8 midwestern dairy states were represented by the samples thus examined. These churning were made and examined during the period February 1st to December 31st, 1938. Seven per cent of the total churning examined were 93 score unsalted, about half of which were

unripened unsalted, the remainder being ripened unsalted. As might be expected, keeping quality of ripened unsalted was found to be superior to the unripened unsalted. In general it was found that when cheesy or putrid type flavors were encountered in unripened unsalted the flavor appeared very soon after manufacture. In several instances butter made from first quality cream developed this flavor before it had left the creamery. Rancid flavor development in unsalted butter usually developed more slowly. As will be seen from table 1, putrid or cheesy flavors in unsalted were usually very definite when encountered on keeping quality tests. Rancid flavors, however, occurred in varying degrees of intensity and somewhat less rapidly though the defect is doubtless equally serious in unsalted butter and apparently occurs more commonly.

Approximately 70 per cent of the churnings examined were 92 and 93 score quality salted butter, there being slightly more 93 score than 92 score. Approximately 7 per cent of the total samples were unsalted butter, the remaining 23 per cent representing 91 scores and below.

All churnings were from weekly shipments of fresh butter and were graded by an official government grader upon arrival. In examination of keeping quality tests after incubation no effort was made to place a numerical score upon the samples. All samples which did not retain their sale value according to original grade were classified as definitely off and unsatisfactory. A surprisingly close correlation between keeping quality tests and subsequent difficulty with the churnings tested was noted.

Where a defect was only slightly indicated after completion of the holding test, subsequent difficulty with the butter was very rarely encountered.

From table 1 it is plainly evident that putrid-cheesy type flavor development was by far the most frequently encountered keeping quality difficulty in salted butter. Tests indicated that with 76.62 per cent of the samples which showed unfavorable keeping quality this type of flavor and odor development was the cause. In addition, when this development occurred almost two out of three cases developed the defect to a very marked degree. The defect was as pronounced in low scoring undergrade churnings as it was in top grades.

Some investigators have classed cheesy flavors according to types of cheese which they resemble. Molds have been found to produce roquefort-like flavors in butter. Bacteria have been found to cause limburger or putrid-type flavors as well as cheddar cheese flavors of varying intensity. The terms putrid or cheesy as used in the present study indicate all types of cheesy flavor development but by far the largest proportion of these flavors encountered were of the putrid or limburger type, which are commonly referred to by the trade as proteolytic or protein decomposition flavors. In general it has been found that organisms commonly encoun-

TABLE I  
*Distribution of samples which developed unfavorable keeping quality according to flavor development and original score.*  
*Per cent of total spoilage for each grade of butter*

tered where unfavorable keeping quality is concerned are of a type easily killed by ordinary pasteurization treatment of cream. Species of *Achromobacter* (1) and *Pseudomonas* (5) organisms have been frequently found in samples of butter having poor keeping quality. No cases of butter spoilage traceable to heat resistant (spore forming) bacteria have been encountered in the present study.

#### SOURCES OF BUTTER SPOILAGE ORGANISMS

The most common and also the most serious butter spoilage concerned in the current study were traceable to contamination from the creamery water supply. Practically without exception, water contamination was responsible for typically putrid or limburger type flavor and odor development in both salted and unsalted butter. In creameries where the finest quality of cream was used and excellent pasteurization practice and sanitary practice on equipment was employed, water was repeatedly found to be the sole source of keeping quality difficulty. In such cases the common procedure was to submit water samples for bacteriological examination and to simultaneously inspect water tank systems, water pumps and wells. As a precautionary measure, water tanks were cleaned and the well, pipe lines, and tanks given a chlorine treatment using chlorinated lime or calcium hypochlorite compounds. It was found that in some cases treatment of the water tanks eliminated the difficulty without further changes. In these cases accumulation of relatively small amounts of sediment of a flocculent nature in the bottom of the tanks which gave off small amounts of gas were indicated as causing the contamination of the butter. Here also it was found that the water delivered from the pump was entirely satisfactory but was contaminated in passing through the storage tanks.

Another group of cases of water contamination was found where breaks in the well casing or drainage entering from the top of the well was responsible. In these instances steps were taken to render the casing tight and to seal off the upper end in such a way that liquid from condensation from the pump or moisture from the floor could not gain entrance to the casing. In addition, in order to disinfect the well itself a quantity of chlorinated lime mixed with thirty parts water was added to the well through the top of the casing. This mixture was allowed to remain in the well for 8 to 10 hours, then 300 to 400 gallons of water were pumped from the well through all outlets and into the storage tank. The pump was then operated until no chlorine odor was perceptible, after which the water was directed to usual use. In cases where localized contamination was encountered, this treatment proved effective in eliminating keeping quality difficulty.

Other instances of water contamination involving continuous seepage entering the water underground were encountered which did not respond

to well treatment. In such cases continuous chlorination by means of mechanical hypochlorite feeders were found effective. Several cases of water difficulty were encountered where creameries located in small municipalities did not have their own wells and were dependent upon the municipal water supply, which was not chlorinated. In such cases water treatment was accomplished by addition of measured amounts of hypochlorite to definite volumes of water placed in a cream vat or galvanized iron tank. This method was effective in eliminating defective keeping quality due to putrid flavor development in all of four such cases encountered.

Samples of butter from two creameries indicated as having water contamination difficulty due to continuous seepage to the well were submitted to laboratory examination and were found to contain *Achromobacter putrefaciens*.<sup>1</sup> This type of spoilage and in general spoilage traceable to water contamination was quite characteristic of putrid or limburger cheese type flavor. Consequently as this study progressed, the writer came to look for water contamination possibilities when this type of flavor development occurred and to apply chlorine treatment where indications of water contamination were found. Very gratifying results in eliminating keeping quality difficulty were attained by this approach to the problem.

Whether the addition of small amounts of chlorine to water used for washing butter are in any way detrimental to the product has not, as far as the writer is aware, been established. It is a well known fact, however, that many large creameries and dairies located in large cities constantly use water from municipal sources which contains from 0.1 to 0.5 parts per million of available chlorine. In the present study concentrations of from 0.2 to 5 parts per million of available chlorine were employed, the amount of chlorine used being proportioned to the contact period and to the pollution hazard. It was established that the official score was not impaired by the use of chlorine in water nor was there an instance of off flavor development on keeping quality tests in the case of butter made using chlorinated water which would indicate chlorine as having an effect in butter deterioration. The highest concentration tried was approximately 10 parts per million and even in this instance there was no apparent effect on the flavor score or the keeping quality of the butter. Occasional cases of cheesy type flavor development were traced to churning and vats, but in these instances the flavor development was neither as definite nor as characteristic as was the case when water contamination was involved.

The rancid flavor developments encountered were largely of the typically butyric or hydrolytic type. In these cases the churning were found to be the most consistent sources of difficulty. In most cases where the churn was indicated it was found that there was also trouble due to stickiness. This was corrected by careful attention to the method of wash-

<sup>1</sup> Samples examined by Dr. B. W. Hammer and Associates at Iowa State College.

ing the churn and by careful attention to temperature of the water used in washing. A practice which gives excellent results is to rinse the churn with water at 100° F. to 120° F. after use to remove any small amounts of butter. The churn is then rapidly filled about 1/3 full of water at 185° F. and revolved in high gear for 20 to 30 minutes, drained quickly and allowed to dry as quickly as possible. It is highly important that the water be at least 160° F. when the churn is drained after washing. Churns washed daily by this method have been found to be kept in excellent condition and difficulty from sticking is practically absent. This treatment does not seem to injure the wood as churns handled in this manner have been known to give satisfactory service for many years. The character of water with respect to hardness in some cases causes accumulation of scale which must be removed occasionally to prevent sticking. Packing glands on churns were found to be kept free of excessive contamination by the above practice in churn sanitation.

A few instances of rancid flavor development were traced to breaks in vat linings, covers and in vat coils. Only in extreme cases were packings on vats used for "holder" type pasteurization found to be in questionable condition. This may be largely due to the fact that a temperature of 165° F. for 30 minutes was commonly used in pasteurization, thereby giving the packing glands a considerable heat treatment. Only when the coil packings were leaking badly or nearly worn out was difficulty from this source indicated.

Considerable attention has been given to so-called sanitary pipe lines and cream pumps as keeping quality hazards in recent years. Properly handled, this equipment should never give trouble or excessive contamination. Some methods of handling this equipment, however, are open to criticism. In the present study, when difficulty was traced to this source, it was found that the reason was not a lack of cleanliness of the equipment. The most desirable practice in this regard was to arrange the system so that only one vat could be connected to the pump at any one time. Thorough scrubbing of the pipes and pumps after using, assembling just prior to use, and sterilizing the assembled system with "live" steam before use was found to be the best method of treatment. Certain types of cream pumps, namely those positive pumps employing oil cups on the inner side of the packing gland, were found to require special attention in sterilizing. The oil cup was discarded and replaced with a metal plug or petcock which was opened when the system was steamed. This practice was found to maintain these pumps in good sanitary condition. When such pumps are used for cream there is no need of the oil cup as butterfat in the cream gives adequate lubrication.

The occurrence of moldy, stale, tallowy and miscellaneous flavors were quite uncommon and were usually found to occur to only a slight degree.

None of these flavors were found to occur consistently in the butter from any single creamery. In contrast, the flavors of the putrid, cheesy or rancid types were found to occur regularly in specific creameries and when eliminated did not reoccur.

For the purpose of illustration specific cases of keeping quality difficulty are subsequently outlined.

#### *Creamery A*

A creamery producing more than 75 per cent of 93 score unripened salted butter was found to have occasional difficulty with putrid flavor development. A complete inspection on all equipment revealed satisfactory conditions and satisfactory processing practice. Cleaning and sterilizing the water tank and careful attention to workmanship were the recommendations made and the trouble disappeared. About one month later the plant began manufacturing unsalted butter and the first shipment revealed several churning which developed putrid flavor and odor. The well was treated immediately with chlorine by adding 3 lbs. of chlorinated lime (24 per cent available chlorine) mixed in 20 gallons of water to the well casing.<sup>2</sup> This mixture was followed by the addition of about 100 gallons of water.

A small amount of water was then pumped through the piping system leaving all outlets open. The well was then allowed to remain undisturbed for 10 hours. After this, sufficient water was pumped to render the flow free of lime. About 400 gallons accomplished this, after which the water was pumped to the storage tank—the tanks having been cleaned and sterilized previously. Immediately following this treatment, keeping quality difficulty in the butter completely disappeared and in more than 10 months no further occurrence of defective keeping quality has appeared. Earlier efforts to eliminate the trouble by simply treating the tanks had proven unsuccessful.

In this case it is thought that accumulation of material above the water pipe level in the well casing was responsible for the trouble, the water level remaining quite a distance above the pipe intake even though the pump was in operation. Stopping and starting the pump, of course, caused a change in the height of water in the casing. The addition of the chlorine mixture is assumed to have affected sterilization of the casing itself and, in addition, to have carried down accumulated solids by mechanical action thus removing a hazard to butter keeping quality.

#### *Creamery B*

This case involved a creamery which was entirely dependent upon a small municipal water supply which was not chemically treated, but which

<sup>2</sup> Recommended and successfully used in treatment of wells at creameries, food plants, and canneries by G. A. Vacha, Chief Bacteriologist of the Minn. Dept. of Agr., Dairy and Food.

was taken from a 200 ft. well. The creamery manufactures a high percentage of 93 score butter and regularly maintains a salt content of 2.0 per cent to 2.3 per cent by analysis. A sudden appearance of a large proportion of unfavorable keeping quality tests showing typical putrid flavor development occurred. The writer visited the plant immediately and instituted a practice of treating all water used in connection with the pasteurized cream and the butter with one part per million of available chlorine. This was done by measuring water into a clean vat and adding measured amounts of calcium hypochlorite powder. Water used in washing the butter and in standardizing the moisture content of the butter was taken from this vat. The above procedure has since been carefully followed out and no further occurrence of defective keeping quality has been encountered. At the time the treatment was begun water samples were submitted to the state bacteriological laboratory. These samples, representing the water as drawn from the city lines at the creamery, were reported as being very unsatisfactory for use in butter due to the presence of excessive numbers of bacteria and in particular large numbers of proteolytic bacteria as measured by plate counts on skimmilk agar.

#### *Creamery C*

This case involves a creamery where the water supply was found to be affected by continuous underground seepage apparently caused by proximity of swampy land and the use of a large cess-pool for sewage disposal some years previous. At any rate, a new and deeper well was installed at the first appearance of difficulty. This installation was effective only temporarily, the trouble again appearing two months later. Routine bacteriological examination of the water directly from the well failed to show appreciable numbers of bacteria. However, treatment of the water used for each churning was the only method whereby the keeping quality of the butter could be maintained. Samples of water drawn on seven consecutive days failed to yield significantly high bacteria or yeast and mold counts. On the basis of these findings a few churnings of butter were made without the use of treated water. Immediately the trouble with keeping quality reappeared. A mechanical hypochlorite solution feeder was then installed and adjusted to deliver water containing 3 p.p.m. of available chlorine. This apparatus has been maintained in continuous operation and no occurrence of unfavorable keeping quality has appeared since that time. A number of other instances of application of regular chlorination of water have proven equally beneficial. It may be added that bacteriological examination of water even when excessive bacteria counts were obtained yielded negative tests for colon-typoid type organisms in all but one of the cases encountered.

## WATER PURITY

When a new well is installed it is very commonly assumed that depth and absence of conspicuous pollution possibilities will render the water obtained satisfactory. As previously pointed out the presence of colon group organisms was detected in only one of the cases studied. On this basis then the water would be considered quite satisfactory for use from a public health standpoint. However, it is evident that spoilage can be carried by water even though public health authorities find it satisfactory. Unquestionably several factors contribute to the bacteriological condition of well water. During the period the current observations were made the mid-west dairy section received an abundant rainfall, which followed several years of relatively little rainfall. Fluctuations in the ground water level and the geologic makeup of subsurface strata are factors which doubtless effect the drainage of surface water to subterranean water levels. Periods of relative drought followed by periods of excessive rainfall are known to effect the pressure on ground water. Increases in pressure cause an increase in the amount of suspended matter water will carry. It is by no means beyond possibility that the amount of rainfall can be correlated to some degree with the presence of excessive numbers of micro-organisms in well water, particularly from comparatively shallow wells. Where the subsurface strata is predominantly sandy and the water table is relatively near the surface, there are good possibilities for contamination from surface drainage. In other areas where the substrata is predominantly limestone surface drainage is afforded relatively little filtration and thus may prove extremely important in water purity. Some suggestions are thus evident with respect to previously reported spasmodic and epidemic appearance of putrid or limburger type spoilage in butter.

There may be methods of water treatment which are more readily adaptable to use in small creameries than chlorination. However, the general use of chlorine and hypochlorite compounds in water treatment and its low cost appears to give the logical answer to water purity problems.

There are several methods which might be used in treating water against spoilage organisms. These include the ozone process, ultra violet treatment, the "electro katadyn" process involving ionic silver and filtration by means of a special filter for removing organisms. Very limited information is available on these methods and even less is known concerning their suitability for creamery use.

Pasteurization of water has previously been successfully used in creamery water treatment. This method, however, is somewhat cumbersome and quite expensive compared to chlorine treatment and if practiced regularly requires special equipment or considerable inconvenience. While there is the possibility that chlorine in water might have an effect on butter,

the use of small concentrations (0.2 to 5 p.p.m.) have not in the present study been found to effect the flavor or sale value of the product, but on the contrary have enhanced the value of butter supplies where unfavorable keeping quality was experienced prior to water treatment. In this connection Hunziker (5) has indicated that chlorine concentrations of 25 to 35 p.p.m. do not injure the quality of butter.

According to present information there is no reason to fear detrimental effects of small amounts of chlorine used in wash water upon the resulting butter. It may be added that the writer has repeatedly made colorimetric tests for available chlorine on wash water drained from the churn and was unable to detect any free chlorine even though the wash water before being placed in the churn contained 5 p.p.m. of residual available chlorine. It appears that the buttermilk diluted out by the wash water effects dissipation of the available chlorine immediately as the chlorinated water enters the churn.

#### RANCIDITY IN BUTTER

Several cases of rancid flavor development in butter submitted to keeping quality tests were encountered. The common appearance of one, two or three samples showing this defect found in weekly shipments was the rule. In one case, however, a very serious outbreak of rancidity development where virtually every churning in an entire shipment was defective in keeping quality was encountered. This was subsequently traced to a sticky churn and a defective packing gland on the cream pump. Another instance of serious rancidity development was subsequently traced to vats in need of repair. In another case where only a very occasional case of rancidity occurred, it was found that each time the churning could be traced back to one particular vat. Inspection of this vat revealed a broken cover lining which was immediately repaired. This done, no further cases of this flavor development occurred. In a few instances cheesy type flavors were occasionally found where outbreaks of rancidity were encountered. These were usually far milder and less pronounced than was the case when cheesiness caused from water contamination occurred.

As a result of the foregoing experience, it may be indicated that where typically putrid or limburger type flavor development occurs in butter held on keeping quality tests, an impure water supply is commonly the ultimate source of the trouble. Where rancidity is encountered the most likely source is the sanitary condition of equipment, particularly the churn, with the vats, cream pumps and pipe lines next in importance in the order named.

Other types of flavor developments did not occur in any significant frequency but were limited largely to butter scoring 91 or less when fresh (see table 1). Two cases of mold development were traced to storage of butter in tubs in a wet cooler. In one of these, failure to sterilize parch-

ment liners was a major contributing cause. Stale flavors in many cases were found to a very slight degree in fresh butter and were slightly intensified in holding. Observation of the held samples at approximately room temperature may have been partially responsible for this condition. Occurrence of tallowy flavors was relatively rare and when found these flavors were present only to a slight degree.

For the most part butter represented by the samples examined was marketed as fresh butter and was consumed within six weeks from the date of churning. Butter which was stored was selected on the basis of creameries whose keeping quality records were satisfactory. After 3 to 4 months storage at -10° F. keeping quality tests were satisfactory on both salted and unripened unsalted butter.

While the keeping quality test employed herein may leave some points to be desired, there is no question as to its value in commercial practice. It is not practical in many instances to await results of the test before marketing any given lot of butter. Nevertheless an early knowledge of a tendency for butter from any source to develop unfavorable keeping quality is of very material value in preventing excessive losses as corrective measures can be brought to bear at the earliest moment. This test may well occupy a position in the butter industry similar to that held by the bacteria count of milk in that although the product may be disposed of before results of the examination are known, definite benefits of the tests on subsequent production are forthcoming before an appreciable amount of the product is placed in trade channels. The keeping quality test for butter can be conducted at the point of manufacture with no special equipment thus bringing practical bacteriological control methods to the creamery itself. Undoubtedly this test is one of the major contributions to the butter industry in recent years and bids fair to find increasingly greater application.

#### SUMMARY

The results of keeping quality determinations by means of holding parchment-wrapped samples of commercial butter at 68° F. to 70° F. for seven days are presented and discussed. Twenty-two thousand sixty churningings of commercial butter were submitted to examination and personal visits were made to creameries whose butter showed unfavorable keeping quality. Putrid and cheesy type flavor development was found to be the most frequently encountered keeping quality difficulty. The major cause of this difficulty was traced to contamination of the creamery water supply with spoilage types of bacteria. Rancid flavor development was also found to be an important cause of defective keeping quality. This defect was most frequently traceable to equipment, notably churns, vats, sanitary pipe lines and cream pumps. Comparatively few cases of mold, staleness or tallowy flavor development were encountered.

Defective keeping quality was eliminated in cases of water contamination by chlorine treatment using hypochlorites. Repairs in equipment and changes in plant practice were found to eliminate rancid flavor development.

#### ACKNOWLEDGMENTS

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# EXTREME RARITY OF CANCER IN THE COW'S UDDER: A NEGATIVE FINDING OF VITAL INTEREST TO THE DAIRY INDUSTRY AND TO THE CONSUMER

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In connection with the investigations in the Bureau of Dairy Industry of the interrelation between conformation, anatomy and producing ability of cows, the udders of 313 cows of lactating age, and of 105 heifers and freemartins, have been filled with formalin, frozen, cut into sections, the sections photographed and otherwise studied in detail. This prescribed program brings to light any lesions, growths or other abnormalities that these udders may contain. Many of the lesions that have been found are thought to have resulted from mastitis but in no case has a cancerous growth been found. This paper describes unusual lesions that were found in two udders and discusses the highly important subject of the cow's apparent lack of susceptibility to mammary cancer.

Cow No. 656 made a 365-day production record at 2 years 4 months of 9,713 pounds of milk and 474 pounds of butterfat. There is no record of any udder trouble that required treatment during the first three lactation periods. During the fifth month of the fourth lactation it was noted on one occasion that the udder was tender and the right front quarter was enlarged, but this condition apparently disappeared in a short time.

The cow commenced her fifth lactation with a heavy milk flow. For the first 89 days the average daily milk production was 49 pounds. During the fourth month of lactation, on June 28, 1932, the right front quarter was reported as being swollen and hot. During the fifth month of lactation some indications of mastitis were noted in the left side of the udder, and at approximately 6 months after calving the right front quarter was hard and no milk was obtainable from it. This condition continued during the remainder of the lactation. After the disturbance in June the cow's total milk production was greatly reduced. Throughout the sixth lactation period the right front quarter was entirely inactive and her total production was low.

She commenced her seventh lactation period with a high level of production, averaging 48.6 pounds of milk daily during the first 92 days. Milk, which appeared to be normal, was obtained from the right front quarter that had been inactive through part of the fifth and all of the sixth lactation periods. This quarter was still very much enlarged. About 2 weeks after

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calving a trace of flakes was noted by strip-cup examination of milk from the right front quarter. The same condition was noted 4 weeks later. No other disturbance in the udder was recorded until she had been in milk for 105 days, when the previously unaffected right rear quarter became hard, the cow went off feed, and the udder dried up in all quarters. She was slaughtered on the 121st day of the lactation period. There appeared to be no swelling in the left half of the udder, which was rated very high in quality. However, the right front quarter was greatly enlarged and broken away from the abdominal wall and the right rear quarter was enlarged.

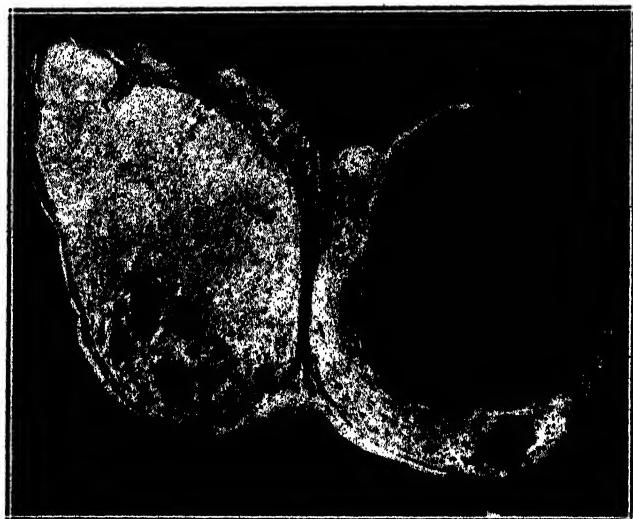


FIG. 1. A section through the front quarters of the udder of cow No. 656 showing an enormous abscess in the right front quarter which is entirely separated from the cistern and the adjacent gland tissue by a wall having approximately the same thickness as the hide covering the udder. The marked enlargement of the right front quarter distorted the udder so that the two front teats were not in the same transverse plane. This accounts for the absence of the left front teat on the section shown in figure 1.

The gross structure of the two front quarters is shown in figure 1. The left quarter does not appear to be abnormal. The right front quarter contained an enormous abscess which apparently had become entirely separated from the gland tissue by a wall having approximately the same thickness as the hide covering the udder. The abscess contained a large quantity of debris. Ventrally and medially to the abscess was an area of gland tissue that looked much the same as the tissue in corresponding areas in the adjacent left front quarter. Apparently this tissue was the source of the milk obtained from this quarter during the early months of the seventh lactation period. The fact that milk secretion was resumed in this quarter after

approximately eighteen months of inactivity during two consecutive lactation periods is very unusual.

The result of the mastitis infection which attacked the right rear quarter about 2 weeks before the cow went dry and was slaughtered, is indicated in figure 2. The gross structure of the tissue in the left rear quarter, like that in the left front quarter, appears to have been unaffected by the disturbance which caused complete cessation of lactation in this udder. It is possible, of course, that a histopathological study of the tissues in the left front and left rear quarters, would show that they had been affected by the infection.

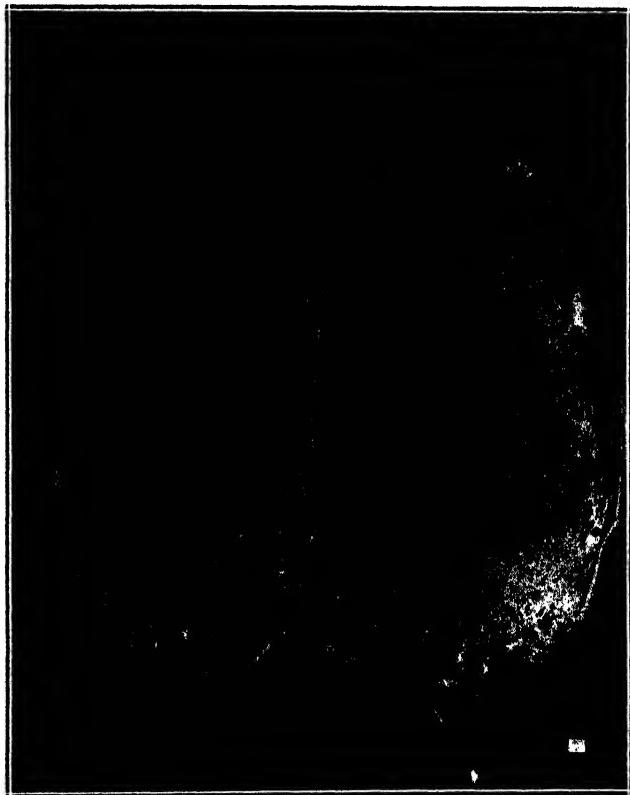


FIG. 2. A section through the rear quarters of the udder of cow No. 656 showing the condition of the tissue in the right rear quarter after an acute attack of mastitis which occurred 3½ months after seventh calving.

Another unusual case is that of cow No. 1011. Some mastitis was noted 16 days after she calved the first time but this condition promptly disappeared. All four quarters were affected by mastitis at approximately 7 and

9 months after first calving but the disturbance did not appear to be marked or persistent. Her record for 305 days at 2 years 7 months of age amounted to 4,782 pounds of milk and 280 pounds of butterfat.

During her second lactation, she completed a 365-day record of 8,343 pounds of milk and 480 pounds of butterfat. Apparently the udder was in good condition except for traces of flakes, on strip-cup examination, in three of the quarters at different times during the last 3 months of this lactation period.

During the third lactation no disturbances were recorded aside from traces of flakes in the milk during the 6th and 7th months. She milked from all four quarters, but the left front and right rear quarters were relatively "light." Her production was comparatively low and she continued in lactation for only 255 days.

On the day of her fourth calving palpation by a pathologist indicated mastitis in all four quarters. Brom-thymol reactions on the same day were positive in all quarters. At weekly strip-cup examinations she showed marked mastitis in the right front quarter, the milk from that quarter becoming watery at 92 days. At 114 days the strip-cup examination indicated very marked mastitis in the left front quarter. The right front quarter was dry and the left front quarter was discharging pus 119 days after calving. She was milked for only 126 days.

About 2 months before her fifth calving the right rear quarter was hard and the tissue was harsh. A month later there was a definite lump in this quarter. The swollen condition continued until calving. Pus was discharged from this quarter before calving. Milk was not obtainable from any of the quarters after calving, even after probing by a veterinarian in an effort to remove or puncture the obstructions which were believed to have been formed in the region of the cisterns.

She was slaughtered July 7, 1936, seven days after calving. Examination of the udder before slaughter indicated that the cisterns in all except the right rear quarter, which was discharging pus, were completely walled off by what appeared to be a layer of fibrous tissue that destroyed all communication between the teat canals and the glandular tissue.

Immediately after the cow was slaughtered the udder was removed and prepared for post-mortem udder studies. The excised udder weighed 82.2 pounds. Because of the unsuccessful attempt to obtain milk by probing before the cow was slaughtered, a sheath knife (*bistoury caché*) was inserted into each teat in an effort to make a passage for the introduction of formalin. The toughness of the fiber is indicated by the fact that the *bistoury* would not go beyond the obstruction until the blade was released. The unsheathed blade, however, cut through a layer that was estimated to be about 1 inch in thickness, after which there was no appreciable resistance. The instrument was inserted approximately 4 inches beyond the point where

the teat was attached to the udder, and rotated with the blade extended. The same procedure was followed in each quarter. The filling of the udder was not entirely successful. It was thought at the time that a sufficient quantity of formalin had been retained to insure adequate preservation. When the frozen udder was sectioned, however, it was found that some of the areas most distant from the teats were not well preserved.

Examination of the sectioned udder showed that none of the four quarters had visible cisterns. The areas normally occupied by cisterns were filled with fibrous tissue (figs. 3 and 4). The marks made by the bistoury are



FIG. 3. Growth of fibrous tissue in the region of the cisterns of both rear quarters of cow No. 1011 had cut off all communication between the teat canal and the gland tissue. Note the channels leading upward from the teats, which presumably were made by the bistoury. Note also the absence of cisterns and the extreme scarcity of ducts in both rear quarters.

visible in figure 3. A heavy-walled abscess was found near the base of the right rear quarter. Apparently this abscess had a small drainage outlet



FIG. 4. Shows an irregular-shaped transverse fissure in the left front quarter of the udder of cow No. 1011. Note also the fibrous tissue growth above the teat, the absence of cisterns and the scarcity of ducts in both front quarters. The dark areas near the top of sections shown in figures 3 and 4 are discolorations resulting from retained blood in the immediate vicinity of the large blood vessels.

into the teat which resulted in the discharge of pus before and after fifth calving.

A peculiar vertical, longitudinal split in the gland tissue was found in the left rear quarter. It commenced somewhat posterior to the rear teat and extended forward for a distance of some 7 or 8 inches, finally became diffused, and disappeared in the connective tissue adjacent to the median septum. The cleft reached a maximum depth of about 8 inches and a width of more than 2 inches. Another pocket or split was found in the left front quarter. In position it was vertical and transverse instead of longitudinal and was located slightly posterior to a vertical plane through the front teats. A general idea of its size, location and general appearance is given by figure 4. It was confined entirely within two of the cut sections, each of which was about 1 inch thick. Except for its color and position it appeared much like the cleft in the left rear quarter but they were several inches apart and there was no detectable connection between them. The cause of the clefts in the two left quarters is not known. Nothing of a similar nature has been found among the hundreds of udders studied. There is no reason to believe that the pressure used in injecting the formalin was responsible. On the other hand, the occurrence of severe mastitis had not been recorded in the left rear quarter, although severe mastitis and suppuration were in evidence in the left front quarter a few days before milking was terminated about 4 months after fourth calving.

Not only were the cisterns completely replaced by fibrous tissue but there was a very marked scarcity of ducts even in the areas near the cisterns where the ducts are generally abundant and often of good size. It was not determined definitely whether the udder was naturally dense throughout or whether the larger ducts, like the cisterns, had been replaced with other tissue. The fact that there was so little fibrous or scar tissue with the exception of the comparatively thin layer above each teat leads one to believe that numerous large ducts were never present in this udder.

Sometimes lesions and other abnormalities are found when udders are sectioned, that were not known or suspected to exist. A number of cases of small abscesses, cysts and deposits of various kinds have been found for which no plausible explanation can be found in the recorded history of the cow.

The two cases that have been discussed and illustrated show what are perhaps the most unusual abnormalities in udder structure that have been found in our work. The remainder of this paper is prompted by negative findings in these udder studies, that is, the absence of cancerous growths.

As stated at the beginning of this paper, 418 udders from animals of all ages have been filled with formalin, frozen, and cut into one-inch sections which were examined for structural make-up and evidences of abnormality, and photographed. The number of gross sections cut from each udder was dependent to a considerable extent on its size and varied from 6 to 46 with an average of 18, a total of more than 7,500 sections. In many cases one-half of the udder was used for the above studies, and the other half was sent to a pathologist who removed samples of the tissue for histological examination. It is particularly noteworthy that in the udders examined no growths or tissue changes that appeared to be of a cancerous nature have been found, though with the technique used a very small growth might have escaped notice.

Cancer usually does not make its appearance either in the human or in other species until after middle life. It is desirable, therefore, that consideration be given to the age of the 313 cows from which the udders included in this study were obtained. It is recognized that a distribution of ages of dairy cows at the time of death does not give a true picture of their normal life span, since most of them are disposed of when their reproductive functions become inactive; when they acquire some communicable disease, such as tuberculosis; or when they otherwise become incapacitated for satisfactory production or for reproduction. A study of the ages of a random sample from nearly 500,000 cows in Dairy Herd Improvement Associations shows that only 4 per cent of the total number were over 10 years, only 1 per cent were over 12 and only one-tenth of 1 per cent were over 15 years of age. It is true that many cows are not slaughtered but die as a result of injury or

disease. On the other hand cows rarely die directly as a result of "old age" and no data are available to show what the actual life span of the cow would be if it were undisturbed by economic factors.

There are a number of lines of reasoning by means of which a purely arbitrary basis can be set up for comparing the life span of the cow with that of the human. At best, however, any basis so set up is only an approximation. The period of fertility might be used as a basis for determining age equivalents. The beginning of the fertile period is fairly definite in each case, but in attempting to establish the age at which cessation of activity in the reproductive functions occurs, one is confronted with a physiological difference which establishes a rather definite termination in the woman but not in the cow. Then too, one might compare the life expectancy at birth, which is approximately 65 years for human white females,<sup>1</sup> with an arbitrary assumed expectancy of 13 years for the cow. On this basis the life span would be 5 times as great for the woman as for the cow and the woman would be 5 times as old as the cow at any given age. This would make the cow of 8 years equivalent in age to the woman of 40.

The following discussion illustrates another basis for estimating age equivalents in the woman and in the cow. In most cases the human female is capable of producing offspring at from 14 to 16 years of age and a woman is usually considered senile if she lives to the age of 80 years. On the other hand a cow usually is capable of delivering offspring at from 1½ to 2 years of age and if she lives to be 18 years of age she is considered to be very old—perhaps comparable in age to the woman at 80. Using 16 years and 2 years respectively as points representing sexual maturity in the woman and in the cow, the subsequent span of life is 64 years in the woman and 16 years in the cow, if 80 years and 18 years respectively are accepted as representing senility. The resulting age equivalents for the woman and for the cow, together with the distribution of the ages at death of the 313 cows whose udders were studied, are given in table 1.

On this basis, as well as on the second one mentioned for comparing life cycles, the cow of 8 years would be equivalent in age to the woman of 40 years. It may, therefore, be significant that 98 of the 313 cows of lactating age from which udders were secured, or 31 per cent, were cows that were over 8 years of age. It appears that a sufficient proportion of the cows studied were past middle life at the time of death to place them in that part of the life cycle in which mammary cancer may be expected to make its appearance in species that are susceptible, yet growths that appeared to be of a cancerous nature were not found in any of the udders.

As a matter of fact, the findings of numerous investigators show that cancer is virtually non-existent in the udder of the cow. According to

<sup>1</sup> Statistical Bulletin, Metropolitan Life Insurance Company, Vol. 20, No. 8, August 1939, p. 2.

TABLE 1

*Age equivalents for woman and for cow, and distribution of ages at death of 313 cows whose udders were studied*

Approximate equivalence in age		Age distribution of the 313 cows whose udders were included in this study		Number of cows
Woman	Cow	Age	Years	
Years	Years	Years	Years	
16 <sup>a</sup>	2 <sup>a</sup>	2 and under	3	23
20	3	3 "	4	49
24	4	4 "	5	42
28	5	5 "	6	27
32	6	6 "	7	39
36	7	7 "	8	35
40	8	8 "	9	24
44	9	9 "	10	18
48	10	10 "	11	23
52	11	11 "	12	20
56	12	12 "	13	8
60	13	13 "	14	2
64	14	14 "	15	
68	15	15 "	16	2
72	16	16 "	17	
76	17	17 "	18	
80 <sup>b</sup>	18 <sup>b</sup>	Over 18		1

<sup>a</sup> Approximate age of sexual maturity.

<sup>b</sup> Approximate age of senility.

Trotter (3) "Cancer of the mamma and uterus is of frequent occurrence in the human female, but in the 300 cases here recorded as occurring in bovines quite a marked difference is noted, none being found in the mamma, and only one in the uterus—a carcinoma." Trout (4) stated that carcinoma of the udder of the milk cow is practically unknown. Drabble (1) quoted Doctor Dodd as referring to "the rarity of cancer in the udder of the cow." Feldman (2), in reporting on nearly 13 million bovines slaughtered subject to meat inspection by the Bureau of Animal Industry in 1930, showed that approximately 1,300 cases of tumorous growths were found but did not indicate that any occurred in the mammary glands. In fact Creech,<sup>2</sup> who reviewed the laboratory findings recorded in a large number of bovine tumors observed in connection with meat inspection activities involving the slaughter of many millions of cattle, over a period of years, has concluded that cancerous growths in the bovine mammary gland are very rare, and that those that have been found apparently originated from carcinoma of the skin and invaded the udder from that source.

It is difficult to understand why cancer, a disease of such high incidence in the mammary glands of humans and in other species should be so nearly non-existent in the cow's udder—particularly in view of the fact that that organ is so highly developed functionally, is often of enormous size, and

<sup>2</sup> Creech, G. T. Veterinarian, Pathological Division, Bureau of Animal Industry, U. S. Department of Agriculture. Correspondence and conversation.

may be subject to friction, irritation and bruises. Nevertheless it is gratifying to know that the gland in which one of the most important foods used for human consumption is produced, is practically free from cancerous growths. This is particularly true in the light of recent investigations reported by Bittner (5), which show that in mice the incidence of breast cancer may be increased in the young of low cancer strains that are allowed to nurse females of a high cancer strain; or may be decreased in the young of high cancer stock that are allowed to nurse females of a low cancer strain. These results are interpreted by Bittner (5) as indicating that "the extra-chromosomal or maternal influence observed in 'breast tumor crosses' may be due to a breast cancer-producing influence' transmitted in the milk of breast tumor stock mothers."

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## HEMOLYTIC STREPTOCOCCI IN RAW MARKET MILK

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The Lancefield technic (1) for the serologic grouping of hemolytic streptococci has furnished, for the first time, a method of detecting significant differences within this group of organisms. This procedure has been used to study streptococci of bovine origin by Plastridge and Hartsell (2), Stableforth (3), Stewart (4) and Edwards (5) among others. Sherman and Niven (6) and Valentine (7) have applied the precipitin method to surveys of the hemolytic streptococci which normally occur in market milk from the state of New York.

The samples tested in the present study were obtained through the kind cooperation of Dr. J. C. Geiger, Director of Public Health of the City and County of San Francisco. All were of raw milk or cream which was to be pasteurized and distributed as a grade A product. During a period of three months, one sample from each of 444 different shippers to this city was tested. Each milk specimen was from a pool of the morning milking from a given herd of 10 to 600 cows. Cream samples were from the pooled lot skimmed at a given plant or station for that morning and hence each was derived from many dairy farms. Samples were collected as a rule from ten gallon shipping tanks. The milk was mixed with a sterile agitator and approximately 200 cc. were transferred to a sterile eight ounce bottle by means of a sterile glass tube. The specimens were kept on ice and were tested within 3 to 10 hours after collection.

Each sample was placed in beef heart infusion agar containing five per cent sheep blood. After 24 to 48 hours at 37° C. colonies which showed any degree of hemolysis but no green discoloration were transferred to beef heart infusion broth containing particles of meat.

All cultures were grouped by the Lancefield precipitin technic using one tube containing 0.1 cc. of antigen, 0.3 cc. of saline and 0.1 cc. of serum for each antiserum tested. Many strains were tested also by the microscopic precipitin method of Brown (8). Tests were made for the fermentation of sorbitol, trehalose, salicin, lactose, raffinose, glycerin and mannite; for the hydrolysis of sodium hippurate and of starch; for the curdling of milk; for the production of soluble hemolysin and for the formation of double zones of hemolysis in blood agar. The procedures are given in detail in another paper (9).

### RESULTS

Hemolytic streptococci were found in 134 of 444 samples of raw market milk from as many different dairy farms and in five of nineteen lots of

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cream. The proportion of samples in which such streptococci were found was slightly higher among samples collected at country skimming and cooling stations than among those from city pasteurization plants. The number of positive specimens from different plants varied from one to eight per twelve samples.

The counts of hemolytic streptococcus colonies from the milk were low with but few exceptions. Most of the samples yielded less than 300 hemolytic streptococci per cubic centimeter, very few showed more than 1,000 per cubic centimeter and none contained over 3,000 per cubic centimeter.

Serologic tests showed that streptococci of group B were virtually always present in positive samples. However, organisms of every group except F were found. Seven samples contained streptococci of more than one group. As shown in table 1, the distribution of the various groups was as follows: group A in three samples, group B in 130, group C in three, group D in two, group E in two, group G in six and group H in one. Four samples contained streptococci which failed to give definite reactions with any antiserum although they showed weak precipitation with serums of group C and G after standing over night.

Biochemical tests as well as serological reactions were carried out with

TABLE 1  
*Serologic grouping of hemolytic streptococci from raw milk and cream samples*

Source	No. tested	No. in which strept. found	Number in which Streptococci found of group:								
			A	B	C	D	E	F	G	H	?
<b>MILK</b>											
Pasteurization plants, San Francisco:											
I	33	18	1	18	0	1	1	0	0	0	0
II	20	9	0	9	0	0	1	0	0	0	0
III	45	6	0	6	0	0	0	0	0	0	0
IV	12	1	0	1	0	0	0	0	0	0	0
V	12	4	0	3	1*	0	0	0	0	0	0
VI-XIX (Plants with less than 10 shippers)	52	12	0	11	1	0	0	0	0	0	0
Total	174	50	1	48	2	1	2	0	0	0	0
<b>Country skimming and cooling stations:</b>											
XX	88	32	0	28	0	0	0	0	6	1	1
XXI	26	16	1	15	0	0	0	0	0	0	0
XXII	22	4	0	4	0	0	0	0	0	0	0
XXIII	50	6	0	6	0	0	0	0	0	0	1
XXIV	78	26	1	24	1	1	0	0	0	0	2
XXV	6	0	0	0	0	0	0	0	0	0	0
Total	270	84	2	77	3	1	0	0	6	1	4
<b>CREAM</b>	19	5	0	5	0	0	0	0	0	0	0
Total—All samples	463	139	3	130	3	2	2	0	6	1	4

\* "Animal pyogenes."

293 cultures from 139 samples of milk or cream. The results, discussed in more detail in another paper (9), are summarized in table 2.

TABLE 2  
*Reactions of hemolytic streptococci from milk*

Sero-logic group	No. of strains tested	No. of samples from which derived	Acid produced from							Milk curdled	Starch hydrolyzed	Sodium hippurate hydrolyzed	Soluble hemolysin formed	Double zones of hemolysis formed
			Sorbitol	Trehalose	Salicin	Lactose	Raffinose	Glycerin	Mannite					
A	3	12	-	-	+	-	-	-	-	-	2+	-	-	-
A	3	1	-	-	161+	4+	-	-	-	-	1-	-	-	-
B	165	99	-	-	-	-	-	-	-	-	158-	-	-	-
B	92	47	-	-	+	+	+	+	-	-	10+	-	-	-
C	6	1	+	-	-	-	-	-	-	-	82-	-	-	-
C	3	1	+	+	-	-	-	-	-	-	-	-	-	-
D	12	12	12	-	-	-	-	-	-	-	-	-	-	-
E	4	4	12	12	-	-	-	-	-	-	-	-	-	-
G	8	6	6	1	+	+	+	+	-	-	-	-	-	-
H	1	1	1	1	1	1	1	1	1	1	1	1	1	1
†	7	4	-	-	-	-	+	5-	-	-	6+	-	-	-
										1-	1-	-	-	-

The reactions of 257 strains of group B were, in general, uniform. None fermented sorbitol, raffinose, or mannite; all produced acid from lactose and all but four fermented trehalose. Seventeen cultures irregularly produced a slight amount of acid from glycerin. Salicin was fermented by 92 strains and was unchanged by 165 of them. Milk was curdled and sodium hippurate was hydrolyzed by all and none attacked starch. All showed at least a trace of soluble hemolysin, 76 gave nearly complete hemolysis and 27 completely hemolyzed red blood cells.

Slightly over half of the members of group B produced double zones of hemolysis in rabbit blood agar plates. After 48 hours at 37° C. and 24 hours in the refrigerator these cultures showed a narrow completely clear zone of hemolysis immediately adjacent to the colony surrounded by a narrower ring of unhemolyzed cells and then by a zone of partial hemolysis somewhat wider than the innermost zone. This double zone phenomenon was observed more often among the salicin fermenting strains (67 of 92) than among those which did not attack this sugar (66 of 165). In sheep blood agar plates, incubated at 37° C. for 48 hours but not refrigerated, double zones were never observed. Under these conditions, about a fourth of the group B streptococci formed clear wide zones of hemolysis but the majority produced narrow areas in which some intact corpuscles were present.

Some samples contained large numbers of streptococci which produced a greenish discoloration of the  $\alpha$  type around surface colonies but which gave typical colorless narrow zones of  $\beta$  hemolysis in deep colonies. Twenty-four of these green-producing colonies were found to be identical both serologically and biochemically with the cultures which showed only  $\beta$  hemolysis.

Usually biochemical tests were made of only one strain of group B per sample, but from 61 samples from two to ten colonies were studied. All from a given specimen gave identical reactions in 39 instances. The only variations found in the cultures in which reactions were not identical were in the fermentation of salicin and in the formation of double zones of hemolysis. From nine samples of milk both salicin-fermenting and salicin-negative colonies were isolated, but these cultures were identical in other respects. Cultures which varied only in their ability to form double zones were obtained from eight samples. In five samples some of the colonies failed to ferment salicin and to show double zones and others possessed both of these properties. Whether such milk actually contained two distinct strains of group B streptococci or whether these differences were due to variability of the cultures is not known.

Only two samples of milk contained typical *Streptococcus pyogenes* of group A. Group B organisms were also present in one of these samples. One specimen contained atypical streptococci which gave strong precipitation with group A antiserum but which hydrolyzed sodium hippurate and failed to ferment any of the sugars tested. These atypical cultures were repeatedly streaked out and repurified but all colonies still gave the same peculiar reactions. No streptococci of other groups could be found in this sample.

The so-called "animal pyogenes" type of group C which ferments sorbitol but not trehalose and which hydrolyzes starch was found in only one lot of milk. The "human" type of group C fermenting trehalose but not sorbitol was isolated from two samples.

Hemolytic cultures of group D which were encountered twice could not be assigned to a species by the tests used. These were not resistant to heating at 60° C. for 30 minutes.

Group E streptococci were found in milk from two dairy farms. The cultural reactions agreed closely with those of the few cultures which have been described. They showed a wide zone of clear cut hemolysis on blood plates and produced potent soluble hemolysin. These were resistant to 60° C. for 30 minutes.

Group G organisms were found in six of the samples. All of these cultures produced acid from lactose but failed to ferment the other sugars used. They were strongly hemolytic both on plates and in the soluble hemolysin test and did not hydrolyze starch or sodium hippurate. The colonies were

not of the "minute" type. A single sample yielded a culture of group H which resembled the group G organisms in every respect except that it failed to produce soluble hemolysin under the conditions of these tests.

#### DISCUSSION

No relationship was evident between the number and kinds of hemolytic streptococci found in the milk and factors such as the sanitary rating of the dairy farm or its geographical location, the distance of shipping or the number or breed of cattle in the herd. Although the country skimming and cooling stations showed a slightly higher proportion of positive samples than the city pasteurization plants, the difference was not significant.

The method used in this survey does not give a true index of the actual number of group B streptococci present because only  $\beta$  hemolytic organisms were considered. It has been established by Stableforth (10) and others that members of this group may be entirely non-hemolytic or may give a greenish discoloration of the  $\alpha$  type. If such colonies had been studied, the proportion of positive samples in all probability would have been higher. Furthermore, non-hemolytic members of groups C, D, G and H, which were not taken into account here, have been described by several investigators.

The incidence of hemolytic streptococci was lower than that reported by others. Valentine (7) obtained hemolytic streptococci from 138 of 244 samples of grade A and B milk. Not more than 30 samples among the 463 in the present series contained streptococci which produced wide zones of hemolysis, whereas Sherman and Niven (6) found 19 such samples among 68 tested or approximately four times as many. They stated that if they had considered narrow zone cultures they would have obtained group B streptococci from nearly every sample, whereas these organisms were found in only about a third of the specimens studied here.

In this survey eight kinds of identifiable hemolytic streptococci were isolated; namely, group A, group B, both the human and animal types of group C, group D, group E, group G and group H. Sherman and Niven (6) found only two kinds of hemolytic streptococci in raw milk; namely group B and the animal type of group C. Valentine (7) found group B, both the animal and human types of group C and group D. The fact that these investigators found fewer varieties might be due to their smaller series of samples or to the presence of larger numbers of other bacteria in the milk. The milk studied here showed extremely low total plate counts and it has frequently been observed that streptococci, if present, can be detected more readily in such milk.

As far as could be determined this is the first time that serologically identified streptococci of groups G and H have been reported in raw milk. Hemolytic group D cultures have been isolated by others from cheese (1)

and from pasteurized milk (6). They are probably present, at least in small numbers, in most raw milk and yet they were found in only two samples in this series and in only seven of Valentine's samples.

Streptococci of the animal type of group C were found in only one sample. Sherman and Niven (6) did not state how often they isolated this organism; but inasmuch as they selected only two or three colonies per sample and described eleven such cultures, the "animal pyogenes" must have been found in at least four or five of their 68 specimens. Valentine (7) reported that this sorbitol fermenting type of group C was found in 57 of 120 samples and hence was more common than group B which was found in only 13 of her specimens.

The group A, G and H strains with atypical cultural reactions are noteworthy because they illustrate the fact that cultures are encountered which can not be identified by any means other than the serologic test. The occurrence of group A cultures which hydrolyze sodium hippurate was of especial interest for such streptococci seem to be rare.

It is not known whether or not all cultures of group B should be given the name *Streptococcus agalactiae* (or *mastitidis*) or whether the group contains more than one species. Stableforth (3), Lancefield (11) and Stewart (4) have found from three to five serologic types within the group. The groups may be subdivided into three varieties according to the reactions on lactose and salicin as proposed by Brown (12), but there is no apparent correlation of the fermentation reactions with the serologic types. The salicin +, lactose + variety has been obtained frequently from both bovine and human sources. Thus far the salicin +, lactose - type has never been isolated and identified by serologic tests from milk. The salicin -, lactose + variety has been found principally in milk but six strains isolated from the throats of children by Plummer (13) were identified as group B by Brown (12) and one culture was obtained from urine by the present writers (9). The available evidence suggests that this variety may be of bovine origin. Frost, Gumm and Thomas (14) gave the name *Streptococcus usalignus* to non-salicin fermenting streptococci from milk. Although these were not classified by the precipitin test it is likely, as Sherman (15) and Brown (12) have pointed out, that they belonged to group B. It does not seem justifiable to regard this variety as a species distinct from *Streptococcus agalactiae* merely because it fails to ferment salicin and is rarely found in human beings. It is not known how stable the streptococci are with regard to their ability to attack salicin and it seems quite probable that this may be a variable characteristic.

Both the salicin +, lactose + and the salicin -, lactose + varieties of group B produce mastitis in cattle under natural conditions (12). Little (16) found that the salicin +, lactose - strains from human infections produced mastitis when experimentally introduced. Hence, even if these prove to be

exclusively of human origin, it seems possible that they may occasionally be transferred to milk in the same manner as *Streptococcus pyogenes* although this has not been reported as yet.

No cultures resembling the *Streptococcus pseudo-agalactiae* of Plastridge and Hartsell (2) were encountered. They described this species as differing from *Streptococcus agalactiae* only in its usual failure to curdle milk, its occasional reduction of methylene blue and its slight precipitin reactions with antiseraums of both groups B and C but strong reactions with its homologous antiserum. It seems doubtful that this variety should be assigned to a separate species.

Hence the decision as to the further subdivision of group B must await further investigation. It would be just as logical to divide it on the basis of double zone formation as on the basis of reactions with salicin and lactose unless a sound serological, cultural and epidemiologic correlation can be shown. In the present state of confusion an effort toward unification rather than hair-splitting subdivision would seem to be indicated.

Brown (12) has stated that all streptococci which form double zones of hemolysis in rabbit blood agar belong to group B, but that there are a few strains of group B which do not form double zones. In the present study and in a previous one (9) no double zone streptococci were found which belonged to groups other than group B. However, nearly half of the group B cultures failed to show this phenomenon although the tests were made repeatedly under the same conditions as those of Brown. Hence group B streptococci could not be identified by this means.

Among the cultural reactions employed in this study those which seemed of value as differential criteria were the fermentation of sorbitol, trehalose and salicin and especially the hydrolysis of sodium hippurate. The determination of the final pH of cultures in glucose broth was used in the earlier part of the survey but was later abandoned. It did not seem to be sufficiently reliable inasmuch as there were strains in all groups which reduced the pH to the 4.8 to 4.6 range. There are, of course, many other valuable biochemical and physiologic tests which were not employed herein, but which would have been indispensable in the absence of the group precipitin method of classification.

Both Brown (17) and Sherman and Niven (6) have suggested that the fibrinolytic test of Tillett and Garner (18) would be an aid in studying milk streptococci. By this test organisms of group A and the human types of groups C and G which dissolve human fibrin can be differentiated from all other streptococci thus far studied. These three groups unfortunately are often impossible to distinguish from each other by biochemical means. Sherman's hope that glycerin and starch tests might serve this purpose was not borne out by subsequent studies (9). It would seem then that the fibrinolytic test which is even more time-consuming and technically difficult than the serologic procedure fails to give as definite an identification.

Hence it is advocated that, for studies of milk in which it is necessary to identify hemolytic streptococci, the serologic method should be used as soon as serum is available commercially. The precipitin grouping must be supplemented by fermentation tests with sorbitol to differentiate between animal and human group C types. In order to identify the various species of group D the tests outlined by Sherman (15) must be used. These include the ability to grow at 45° C. or at 10° C.; growth in the presence of 6.5 per cent bile blood agar; the production of ammonia and the heat resistance at 60° C. for 30 minutes. Undoubtedly additional biochemical and physiological methods will be added to this list as further information is obtained.

The fact that high grade milk produced under rigid inspection and, so far as the plate count is concerned, above the standard set for certified milk, may contain hemolytic streptococci of human origin is a strong argument for universal pasteurization even though the number of instances in which such organisms are found is extremely low. In this city all milk including the certified grade must be pasteurized.

#### SUMMARY

A total of 444 samples of raw market milk from as many different dairy farms was examined for the presence of hemolytic streptococci of both wide-zone and narrow-zone types. Such streptococci were found in 134 of these samples and were distributed among the Lancefield serologic groups as follows: group A in three samples, group B in 125, group C of the animal type in one, group C of the human type in two, group D in two, group E in two, group G in six and group H in one. Only four samples contained streptococci which could not be assigned to a group. Nineteen samples of raw cream were tested and group B streptococci were found in five of them. Approximately half of the group B streptococci produced double zones of hemolysis in rabbit blood agar.

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# STUDY OF DAIRY CLEANING PROBLEMS I. FILMS AND DEPOSITS ON HOT-MILK EQUIPMENT

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The removal of films or deposits from dairy equipment used in the heating and holding of milk products often presents a serious problem. Parker and Johnson (1) early directed attention to the practical and scientific aspects of this problem. They defined *milk film* as the deposit which forms on metal heat-transfer surfaces due to the precipitating action of the heat alone. *Milkstone* was defined as the product resulting from the reaction between the milk film as described and the chemical constituents of the water supply and alkaline detergents applied or their end products.

In commercial operations where milk products are heated, particularly above 140° F., by metal heat-transfer surfaces, there appears to be a daily film formation of some extent depending on the thermal differential and the type of equipment. This may vary from an extremely thin, transparent or translucent film appearing as a bluish or brown discoloration when viewed from an angle, on heater plates or tubes, to a heavy cheese-like blanket of milk solids on batch pasteurizing vats in which relatively rapid heating is accomplished by means of a high jacket temperature. Before an effort was made to determine the most suitable detergents or cleaning methods for hot-milk equipment, it was deemed advisable to study further the mechanism of the film formation on metal heat-transfer surfaces and to account, if possible, for some of the differences observed.

## EXPERIMENTS

A simple apparatus was devised by means of which milk could be heated continuously under controlled conditions. The apparatus consisted of a 9-inch length of 2-inch stainless steel tubing stoppered on both ends with rubber stoppers. One stopper contained inlet and outlet tubes of 5 mm. I. D. glass tubing and a thermometer, of which only the bulb was extended on the inside. The inlet tube extended to within one inch of the bottom stopper. The outlet tube was flush with the inner surface of the top-retaining stopper. This assembly was placed vertically in an agitated, gas-heated water bath. The raw milk of 4 per cent fat content, forewarmed to 85° F., was allowed to flow through the tube by gravity, being drawn off at the desired temperature through regulating the flow by means of a stop-cock on the inlet tube.

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*Trial 1.* In this experiment 7 liters of milk, forewarmed to 85° F., were heated to 143° F. in the tube, maintaining the jacket at 180° F. The milk was cooled continuously to 85° F. after passing through the tube and was recirculated continuously. The milk was collected in a graduate and the rate of flow was measured. After the initial quantity of milk in the tube reached 143° F., the time required to heat the first liter portion was 6 minutes. The heating time increased gradually because of film formation on the heating surface until that for the thirteenth liter (the last one) was 12 minutes. At the end of the run the milk remaining in the tube was poured out and a liter of cold water passed through the tube to rinse the deposit. The deposit consisted of milk solids in the form of small craters of cheese-like consistency. These completely covered the upper part of the tube and graded off to a sparse covering of the lower part. Their diameters were about 0.5 to 1.0 mm. When the soft material was scraped off, there was found to be a fairly strong bond between the metal and the milk solids. The tube was cleaned with hot washing powder solution and powdered pumice in preparation for the next trial.

*Trial 2.* In this experiment 7 liters of raw milk were heated to 143° F. with the jacket at 180° F. This milk was drawn off in liter portions but not recirculated. The time required for heating liter portions varied from 7 minutes for the first to 10 minutes for the seventh. The tube was rinsed with a liter of cold water and opened for inspection. The heating surface was quite evenly covered with the small crater-like particles, many of which contained gas bubbles. Furthermore, it was observed during this run that considerable gas was expelled from the milk and appeared at the outlet tube. These observations suggested that the dissolved gases in the milk might play an important part in the formation of this type of heat-precipitation. The deposit was scraped out and analyzed with the following results:

Fat	51.53%
Protein ( $N \times 6.38$ ) .	35.32
Ash	4.77
CaO	2.28
P <sub>2</sub> O <sub>5</sub>	2.10

The tube was cleaned with hot washing powder solution and powdered pumice. Vigorous scouring was required to eliminate the last traces of the deposit which appeared as a bluish discoloration of the surface, discernible only after the milk solids had been removed.

*Trial 3.* In this experiment 7 liters of milk were held under 15-16 mm. (Hg) vacuum at a temperature of about 55° F. for 2 hours. The temperature was elevated then to about 70° F. and the milk allowed to boil gently for 10 minutes. The vacuum was broken then, the milk warmed

to 85° F. and 7 liters heated in the tube, as in previous tests, to 143° F. with a jacket temperature of 180° F. The rate of heat transfer during this run did not vary significantly, 5 minutes being required to heat the first as well as the seventh liter. When the tube was opened, it appeared that there was no deposit whatsoever on the surface; but after rinsing with distilled water and drying in air, it became apparent that a bluish discoloration covered the heat-transfer surface of the tube. When the surface was viewed as nearly as possible at right angles, it appeared as a very thin, translucent film.

*Trial 4.* In this experiment milk was deaerated as in Trial 3 and heated in the tube to 143° F. with the jacket at 200° F. The first liter through required 3 minutes, the seventh 4 minutes. When the tube was rinsed and opened, a few very small craters of cheese-like consistency were present and the whole heat-transfer surface was covered with the bluish discoloration as previously observed. Observation from a nearly perpendicular position showed the surface to be covered with a thin, dense whitish film.

*Trial 5.* In this experiment 7 liters of milk were deaerated continuously by spraying the milk into a vacuum chamber. The milk, heated to 115° F., was drawn into a flask under 60 mm. (Hg) vacuum. The treatment required about 1 hour. Seven liters of the milk were heated as in previous experiments, this time to 170° F. with the jacket at 200° F. The first liter through required 3½ minutes and the seventh 5 minutes. After rinsing with a liter of cold water, the tube was opened. There were none of the precipitated milk solids on the heat-transfer surface. It had only the film appearing as a bluish discoloration when the tube was looked into and as a dense whitish translucent coating when viewed perpendicularly. There appeared to be more of the metallic-like film produced in this test than in the previous ones.

*Trial 6.* As a control to the previous experiment, untreated milk was heated in the tube to 170° F. with the jacket at 200° F. Only 5 liters were run through. The first required 8 minutes, the second 10 minutes, the third 13½ minutes, the fourth 17½ minutes and the fifth 21½ minutes. When the tube was rinsed and opened, a heavy blanket of the cheese-like milk solids was found deposited on the heat-transfer surface.

*Trial 7.* A practical test was made of the principle of high velocity flow against the metal heat-transfer surface as a means of eliminating the heat-precipitation of cheese-like solids due to the action of gas bubbles as previously described. An experimental procedure was being used in the technological laboratory whereby a 40-gallon batch of cold skimmilk was

heated and pasteurized at 203° F. by means of a 60-gallon stainless steel spray-type vat. This procedure was time-consuming and resulted in excessive deposits of milk solids even when low thermal differentials were used. In this test the skimmilk contained in the spray vat at 58° F. was circulated by means of a centrifugal pump through a box-tube heater, containing 6 stainless steel tubes one and one half inches  $\times$  6½ feet, at the rate of 67 g.p.m. or approximate average velocity of 5 feet per second. The temperature of the steam-heated water jacket of the heater at the beginning of the run was 180° F. and was gradually increased to 210° F. at the finish. To heat the 40 gallons of skimmilk from 58° F. to 203° F. required 12 minutes. At the end of the operation the temperature in the heater jacket was dropped rapidly and the circulation stopped. After draining and flushing the tubes with cold water an examination disclosed that there were no cheese-like solids on the heat-transfer surfaces. After the tubes had dried, some of the bluish discoloration or film was found in the tubes. There was surprisingly little of this, however, in comparison with that found in the laboratory experiments previously described. The hot milk was held in the spray vat for 30 minutes at 203° F., maintaining a 1°-2° higher temperature in the heating jacket, after which the milk was cooled and drawn off. The milk was rinsed off with cold water and the vat examined. Along the upper sides was found a trace of the film or discoloration. Cheese-like solids, however, were entirely absent.

#### DISCUSSION

These tests have shown that 2 types of film may be produced on metal heat-transfer surfaces during the processing of milk. One is essentially cheese-like in composition and is believed to result from the super-heating and partial dehydration of the milk films on the surface of the gas bubbles which, in being expelled from the milk, cling to the metal heat-transfer surface. This is the type of film or deposit commonly found in jacketed vats or pans used for heating milk products. Were it not for the sweeping action of high velocity flow in tubular or plate heaters or the action of agitator blades in other types of heaters, it might be expected that more trouble would be encountered with this type of film.

Davies (2) observes the formation of particles, apparently of cheese-like consistency, on the surface of sterilized milk bottles. He attributes this to the precipitation of the proteins because of their concentration at the air/liquid interface of gas bubbles. He states also that slight heat coagulation on hot surfaces is associated with the foaming of fresh milk on boiling and that such coatings have a serious effect on the efficiency of heat exchange in pasteurizing or vacuum pan work.

The other type of film which is produced concomitantly with the cheese-like film or independent of it, according to conditions, is believed to consist

of calcium and magnesium phosphates, citrates and possibly proteinates, precipitated by the heat-transfer surface. Because of the difficulties encountered in procuring a sample of this extremely thin film, no analysis was made. This metallic-like film or discoloration, while it may be almost insignificant in extent after one processing, is believed to be the real precursor of milkstone found on hot-milk equipment. The cheese-like film dissolves readily in alkaline washing solutions, as will be shown in a following report, leaving the metallic-like film or discoloration. This is probably often overlooked in commercial operations until successive processings result in a film of such extent that the presence of milkstone is recognized. From the data presented by Parker and Johnson (1) and Tuckey (3), it is apparent that there are wide variations in the final composition of milkstone from commercial operations depending on such factors as daily cleaning efficiency, type of washing solution, type and hardness of water, and, as indicated in this work, the processing equipment and procedure.

#### SUMMARY AND CONCLUSIONS

1. An apparatus and method was devised for studying the formation of heat-deposited milk films.
2. Two definite types of films were found: one of cheese-like consistency is the result of super-heating and partial dehydration of milk around gas bubbles formed at the heat-transfer surface; the other a thin dense metallic-like film or discoloration is produced concomitantly with the cheese-like deposit, or independent of it if conditions are such that gas bubbles do not form at the heat-transfer surface.
3. It is suggested that the accumulation of thin metallic-like films, due to incomplete cleaning or failure to recognize their presence, is the primary cause of milkstone formation on hot-milk equipment.

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## STUDY OF DAIRY CLEANING PROBLEMS. II. EFFECTIVENESS OF ALKALIES IN REMOVING HEAT-DEPOSITED MILK SOLIDS AND BUTTERFAT FILMS

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Practical experience has shown that a satisfactory detergent for cleaning dairy equipment must be capable of dissolving or removing dried or heat-deposited milk products and be effective in "cutting" or emulsifying fat and grease. Other important attributes are non-corrosiveness to dairy metals and the ability to produce flocculent, non-adhering precipitates of calcium and magnesium salts in natural water or better still to maintain such salts in solution on the alkaline side.

### A. REMOVAL OF HEAT-DEPOSITED MILK SOLIDS

In order to determine the relative effectiveness of the common alkalies in dissolving and removing heat-deposited milk solids, a simple apparatus was devised by means of which a controlled and fairly reproducible deposit could be produced. It consisted of a 9-inch length of 2-inch stainless steel tubing closed at both ends with rubber stoppers. One stopper contained inlet and outlet tubes of 5 mm. I. D. glass tubing and a thermometer, of which only the bulb was extended on the inside. The inlet tube extended to within 1 inch of the bottom stopper. The outlet tube was flush with the inner surface of the retaining stopper. This assembly was placed vertically in an agitated gas-heated water bath held at 180° F.

In all tests 7-liter portions of mixed raw milk of 4 per cent fat content were forewarmed to 85° F. and allowed to flow by gravity through the heater tube. The milk was drawn off at a constant temperature of 143° F., regulating the flow by means of a stop-cock on the inlet tube. This process resulted in the formation of an evenly distributed coating of cheese-like milk solids on the heating surface of the tube. The nature of heat-deposited milk films was discussed in a previous study (1).

To test the ability of alkali solutions to dissolve and/or remove the milk solids, the tube was first rinsed with a liter of cold water to remove the adhering milk. From 10 per cent stock solutions of the alkalies a 500 cc. quantity of test solution of desired concentration was made by dilution with tap water. The solutions were circulated through the tube, using a 2- to 3-inch gravity head, for 30 minutes at 130° F. At the conclusion of the treatment the tube was opened and examined. The solution was rated *good* if the deposit was completely removed, *fair* if the deposit was softened com-

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TABLE I  
Effectiveness of alkalies at various concentrations in removing heat-deposited milk solids

Alkali	Cone.	pH	Phenolphthalein alkalinity (as NaOH)	Methyl Orange alkalinity (as NaOH)	Deposit removal	Per cent	Per cent
						Per cent	Per cent
Trisodium phosphate (con'')	-	0.100	11.4	-	poor	0.026	0.026
,"	,"	0.212	11.7	0.032	fair	0.046	0.046
,"	,"	0.300	11.8	0.041	good	0.075	0.075
Sodium metasilicate (con'')	-	0.060	11.5	0.022	poor	0.026	0.026
,"	,"	0.100	11.7	0.036	fair	0.040	0.040
,"	,"	0.200	12.0	0.073	good	0.078	0.078
Sodium carbonate anhyd. (C.P.)	-	0.063	11.0	0.023	poor	0.048	0.048
,"	,"	0.100	11.1	0.040	fair	0.074	0.074
,"	,"	0.200	11.2	0.075	good	0.146	0.146
Sodium hydroxide (C.P.)	-	0.010	11.4	0.010	poor	0.012	0.012
,"	,"	0.020	11.8	0.020	fair	0.023	0.023
,"	,"	0.030	11.9	0.029	fair	0.033	0.033
,"	,"	0.040	12.0	0.042	good	0.046	0.046

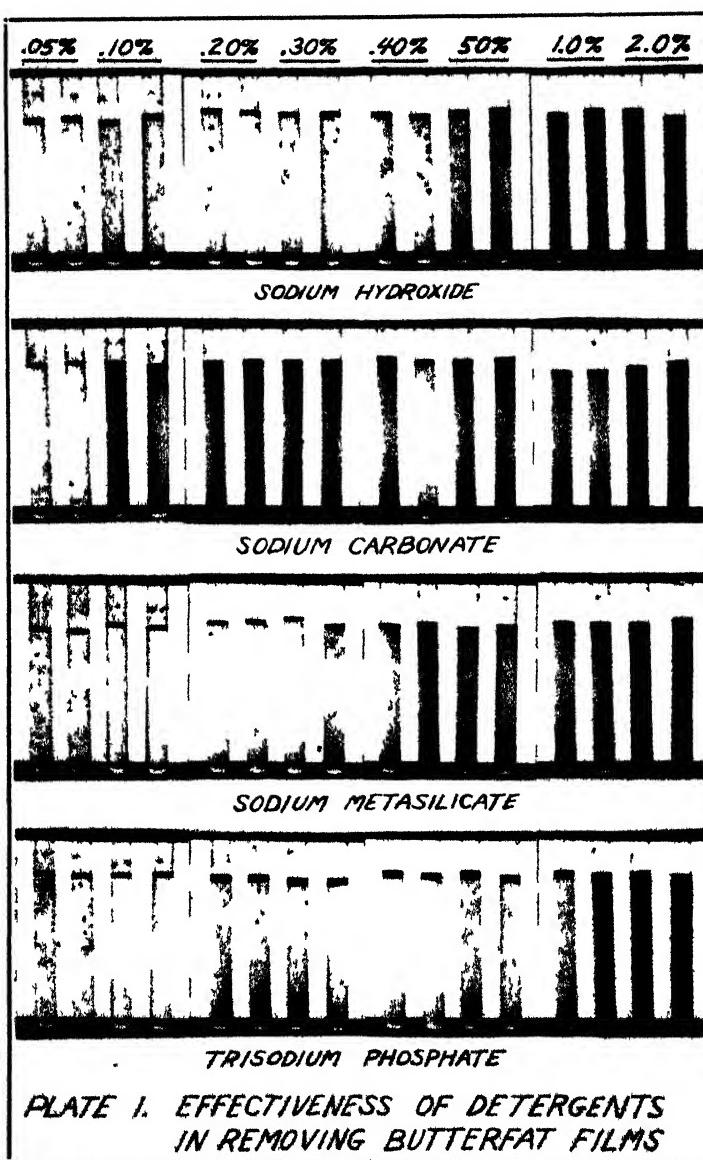
pletely and partially removed, and *poor* if the deposit was not affected or only slightly softened. Between each test the tube was scoured with powdered pumice. Alkalinity by titration and pH by the glass electrode were determined on each solution. The results of typical tests covering the range of effectiveness of the alkalies are shown in table 1.

It will be noted that the approximate minimum effective concentrations were as follows: trisodium phosphate 0.3 per cent, sodium metasilicate 0.2 per cent, sodium carbonate 0.2 per cent and sodium hydroxide 0.04 per cent. As a basis for comparison one solution of each alkali was formulated to produce a phenolphthalein alkalinity of about 0.02 per cent as NaOH. At this alkalinity trisodium phosphate and sodium hydroxide were more effective than sodium metasilicate and sodium carbonate. It is of interest to note that sodium carbonate was effective at a considerably lower pH value than the other alkalies. It should be pointed out that the complete removal of the cheese-like deposit by the alkalies studied did not result in chemically clean metal surfaces. The very slight metallic-like film or discoloration was always found to be present after the cheese-like deposit has been removed. It is believed that this fact is not generally recognized in commercial operations.

#### B. REMOVAL OF BUTTERFAT FILMS

A quantity of dry, clean butterfat was prepared from sweet butter and dyed dark red with oil soluble red dye. New soft-glass culture tubes (15 x 150 mm.) were washed with soap, rinsed, and air dried. The tubes were filled with the melted fat at 130° F., emptied immediately and allowed to drain for 5 minutes in an oven at 130° F. after which they were turned upright and held for an additional 5 minutes. This resulted in the formation of a fairly thin film of fat on the inside of the tube. Immediately after the final holding period the tubes were transferred to a constant temperature water bath at 130° F. where they were immersed to within 2 or 3 cm. of the top. Ten-cc. portions of the various detergent solutions at 130° F. were allowed to drop into the tubes from the wide end of a 10-cc. bulb-type pipette held centrally over the mouths of the tubes. After standing in the bath for 10 minutes the tubes were carefully removed and photographed. Each test was made in duplicate and every precaution was taken in making the tests and photographing the results to insure comparability. This test is a modification of Baker's procedure (2).

A series of tests were made, using pure sodium hydroxide, sodium carbonate (anhyd.), commercial sodium metasilicate, and trisodium phosphate. Tap-water solutions ranging from 0.05 per cent to 2.0 per cent were made from 10 per cent stock solutions of the alkalies. The photographic results are shown in plate 1. It should be pointed out that fat remaining on the glass surface appears as dark globules or patches. The darkness of



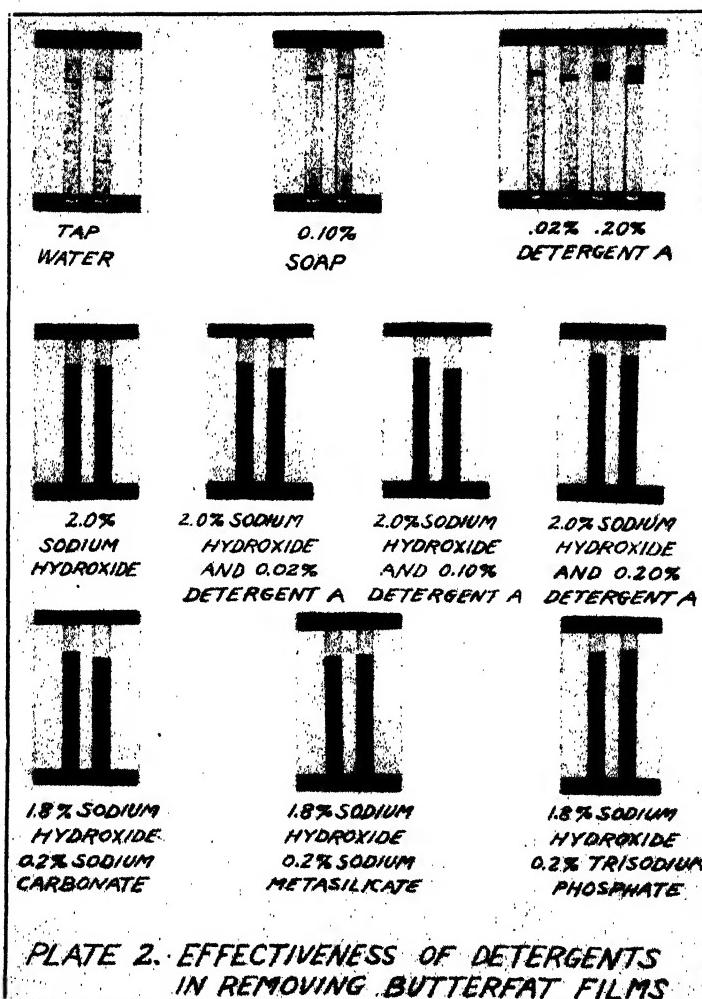
the liquid indicates creaming or emulsification of the fat. It appears that sodium hydroxide alone was not effective at any concentration studied in the removal of butterfat films. This agrees with the experience of many plant operators who have observed poor rinsing or greasy bottles produced by a mechanical bottle washer using caustic soda alone as the detergent. In the range of concentrations generally used for equipment cleaning (0.1 per cent to 0.5 per cent), there seems to be very little difference in the effectiveness of the other three alkalies. The removal of the fat appears to be associated with a greater degree of emulsification in the case of sodium carbonate and sodium metasilicate than in the case of trisodium phosphate but the commercial significance of these small differences is questionable.

In order to study fat removal as related to bottle washing, tap-water solutions of 1.8 per cent sodium hydroxide and 0.2 per cent each of sodium carbonate, sodium metasilicate and trisodium phosphate were tested. Tap-water solutions of sodium hydroxide at 2 per cent with additions of 0.02 per cent, 0.1 per cent and 0.2 per cent of detergent A (a surface-active organic detergent) were tested also. Tap-water solutions of castile soap at 0.1 per cent and detergent A at 0.02 per cent and 0.2 per cent were included for comparison. The photographic results are shown in plate 2. The combination of sodium hydroxide and sodium carbonate did not produce as good fat removal as did the combinations with sodium metasilicate, trisodium phosphate and detergent A. Detergent A alone was very effective in removing the fat, although there was no evidence of emulsification as was the case with the alkalies.

There appears to be a fundamental difference between the activity of sodium metasilicate with sodium hydroxide and that of trisodium phosphate with sodium hydroxide. The metasilicate combination showed a marked "cream" whereas the phosphate combination held the fat at the surface in a more or less continuous fat phase. The latter was true also of the combination of sodium hydroxide and detergent A.

#### SUMMARY AND CONCLUSIONS

1. The approximate minimum concentrations of alkalies effective in removing heat-deposited milk solids were as follows: trisodium phosphate 0.3 per cent, sodium metasilicate 0.2 per cent, sodium carbonate 0.2 per cent and sodium hydroxide 0.04 per cent.
2. At a phenolphthalein alkalinity of approximately 0.02 per cent (as NaOH) trisodium phosphate and sodium hydroxide were more effective than sodium metasilicate and sodium carbonate in removing heat-deposited milk solids. The minimum effective pH values were as follows: sodium carbonate 11.2, trisodium phosphate 11.8, sodium metasilicate 12.0 and sodium hydroxide 12.0.



3. In the removal of butterfat from glass sodium hydroxide was not effective at any concentration from 0.05 per cent to 2.0 per cent. Sodium carbonate, sodium metasilicate, and trisodium phosphate all were fairly effective above 0.1 per cent.

4. A combination of 1.8 per cent sodium hydroxide and 0.2 per cent sodium carbonate was not as effective in fat removal as similar combinations of sodium hydroxide with sodium metasilicate and trisodium phosphate. The sodium metasilicate mixture produced a greater degree of emulsification than did the trisodium phosphate mixture.

5. Combinations of 2.0 per cent sodium hydroxide and 0.1 per cent and 0.2 per cent detergent A (surface-active organic detergent) were effective in fat removal and similar in action to the trisodium phosphate combination. Detergent A alone was effective at 0.2 per cent but did not produce emulsification.

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## American Dairy Science Association Announcements

### THIRTY-FIFTH ANNUAL MEETING

Plans are approaching completion for the housing and entertainment of the members and guests attending the 35th Annual Meeting of the American Dairy Science Association to be held June 24-28, 1940, at Purdue University, West Lafayette, Indiana.

A letter accompanied by a list of housing accommodations and a reservation form has been sent to all members. Accommodations of every kind will be available, including the new Cary Residence Halls, Purdue Memorial Union Club, Hotels in Lafayette, private homes and tourist cabins. To facilitate arrangements, those planning to attend are urgently requested to fill in the reservation form and return it to the Chairman of Registration and Housing before June 1st.

SEE THE JUNE ISSUE OF THE JOURNAL  
FOR THE COMPLETE PROGRAM AND ABSTRACTS

Plan now to attend this fine meeting. Bring the family. A program of entertainment is being arranged for the ladies and children. Tours, coffee hours, luncheon bridge and recreational activities are on the program. In addition there are fine recreational facilities available for everyone, golf, tennis, swimming and bowling.

See Purdue University—located on the banks of the Wabash—120 miles from Chicago, 60 miles from Indianapolis, fine roads in all directions.

*Send in your reservations now!*



# JOURNAL OF DAIRY SCIENCE

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## PROGRAM

THIRTY-FIFTH ANNUAL MEETING  
OF THE  
AMERICAN DAIRY SCIENCE ASSOCIATION

PURDUE UNIVERSITY  
WEST LAFAYETTE, INDIANA

JUNE 24-28, 1940

### PROGRAM COMMITTEE

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## AMERICAN DAIRY SCIENCE ASSOCIATION

*The Thirty-fifth Annual Meeting*

West Lafayette, Indiana, June 24-28, 1940



PURDUE MEMORIAL UNION BUILDING  
HEADQUARTERS OF THE MEETINGS

## GENERAL PROGRAM

*Monday, June 24*

- 9 A.M.-5 P.M. General Registration and Room Registration, Purdue Memorial Union Building.
- 2-4 P.M. Dairy Products Judging Conference for Coaches and Instructors. Smith Hall. K. C. Boxell and R. E. Roberts, in charge.
- 1-3 P.M. Open Meeting of Special Committee to Study the Work of the College Feed Conference Board. Purdue Memorial Union Building, Room 350. Paper—"The history of the open formula and the College Feed Conference Board," E. S. Savage, Cornell University.
- 3-4:30 P.M. Tour of Dairy Barns. J. H. Hilton, in charge.
- 8 P.M. Board of Directors Meeting. Purdue Memorial Union Building, Room 263.
- 8 P.M. Family Get-Together. South Cary Hall Lounges.

*Tuesday, June 25*

ALL SECTIONAL AND COMMITTEE MEETINGS WILL BE HELD IN THE PURDUE MEMORIAL UNION BUILDING. ROOM IS DESIGNATED AFTER EACH MEETING.

- 8 A.M.-9 P.M. General Registration and Room Registration.

9:30 A.M.-12 NOON	Opening Session, North Ballroom, Room 287, E. S. Guthrie, presiding. Address of Welcome: E. C. Elliott, President, Purdue University.
	Response and Address: E. S. Guthrie, President, American Dairy Science Association.
	The Milkfat Globule Paul F. Sharp, Cornell University.
1:30 P.M.-4 P.M.	Manufacturing Section. Sectional Meeting, Room 340.
1:30 P.M.-4 P.M.	Production and Extension Sections combined. A. H. Kuhlman, presiding, Room 350.
4 P.M.	SECTIONAL COMMITTEE MEETINGS. Committee chairmen secure room assignments at registration desk. Extension Section. Manufacturing Section. Production Section.
8 P.M.	Reception, Rooms 340 and 350.

*Wednesday, June 26*

8 A.M.-5 P.M.	General Registration and Room Registration.
8 A.M.-9 A.M.	SECTIONAL COMMITTEE MEETINGS. Extension Section. Manufacturing Section. Production Section.
9 A.M.-11:30 A.M.	SECTIONAL MEETINGS. Extension Section, Room 363. Manufacturing Section, Room 340. Production Section, Room 350.
8:30 A.M.-11:45 A.M.	Complimentary Luncheon, Livestock Pavilion.
12 NOON	SECTIONAL MEETINGS. Extension Section, Room 363. Manufacturing Section, Room 340. Production Section, Room 350.
1:30 P.M.-4 P.M.	SECTIONAL MEETINGS. Extension Section, Room 363. Manufacturing Section, Room 340. Production Section, Room 350.
1:30 P.M.-5 P.M.	SECTIONAL BUSINESS MEETINGS. (Use same rooms as for Sectional Meetings.)
4 P.M.-5 P.M.	Entertainment. Music Hall.
8 P.M.	

*Thursday, June 27*

8 A.M.-9 A.M.	SECTIONAL COMMITTEE MEETINGS. Extension Section. Manufacturing Section. Production Section.
9 A.M.-11:30 A.M.	SECTIONAL MEETINGS. Extension Section, Rooms 230-257. Manufacturing Section, Room 340.

8:30 A.M.-12 NOON	Production Section, Room 350.
11:30 A.M.-1 P.M.	Lunch and Picture.
1 P.M.-4 P.M.	<b>SECTIONAL MEETINGS.</b> Extension Section, Room 363. Manufacturing Section, Room 340. Production Section, Room 350.
4 P.M.	<b>GENERAL SESSION</b> , E. S. Guthrie, presiding, North Ballroom. Program Commemorating the "50th Anniversary of the Babcock Test." 1. Dr. Babcock, the Scientist. Professor E. B. Hart. 2. Dr. Babcock, the Man. Dr. Gustav Bohstedt.
6:30 P.M.	Annual Association Banquet. Presentation of Borden Awards.

*Friday, June 28*

9 A.M.-11 A.M.	GENERAL SESSION, South Ballroom, Room 237.
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**SECTIONAL PROGRAMS****EXTENSION SECTION**

Tuesday, June 25, 1:30-4:00 P.M.

Room 350, Union Building

A. H. KUHLMAN, *Presiding**Joint Session With Production Section*

E1—The nation-wide D.H.I.A. proved sire program. J. F. Kendrick, Bureau of Dairy Industry.

E2—The importance of selective registration to the dairy industry. Lynn Copeland, The American Jersey Cattle Club.

*Symposium on Artificial Insemination*Discussion Leader: E. J. PERRY, *Chairman*, Better Sire Committee, New Jersey

P1—Vitamin C for sterile and partially sterile sires. Paul H. Phillips and Henry A. Lardy, University of Wisconsin.

P2—The storage of bull spermatozoa. H. A. Herman and Eric W. Swanson, University of Missouri.

P3—Some observations on the morphological variations in the spermatozoa of dairy bulls. Eric W. Swanson and H. A. Herman, University of Missouri.

P4—Fecundity and certain other characteristics of fresh and stored bovine semen. H. P. Davis, G. W. Trimberger, Gravers K. L. Underbjerg, University of Nebraska.

*Discussion Panel*

Phillips—Herman—Davis—Bartlett—Hutton

Wednesday, June 26, 9:00—11:30 A.M.

Room 363

R. G. CONNELLY, *Chairman*

- E3—Utilization of proved sires and sons of proved sires. Floyd Arnold, Iowa.
- E4—An Appraisal of Cooperative Artificial Insemination to Date. Stanley Brownell, Cornell University.
- E5—Observations in the care and management of dairy bulls. R. R. Welch, Pennsylvania State College.
- E6—Recommended methods of feeding and management for keeping sires fit. E. E. Heizer, University of Wisconsin.

*Panel Discussion on Breeding Problems*

Leader: Floyd Arnold

Panel Members: Arnold—Cash—Welch—Heizer—Bartlett

Wednesday, June 26, 1:30—4:00 P.M.

Room 363

R. G. CONNELLY, *Chairman*

- E7—Report of feeding committee. A. R. Merrill, Chairman, Connecticut State College.
- E8—Suggestions for making better use of D.H.I.A. feed records. R. G. Connelly, Virginia Polytechnic Institute.
- E9—Accuracy and use of D.H.I.A. feed records. C. G. Cushman, Clemson Agricultural College.
- E10—A method for determining feeding levels in D.H.I.A. herds. W. T. Crandall, Cornell University.
- E11—Report of testing committee. R. C. Jones, U.S.D.A., Chairman.

Thursday, June 27, 9:00—11:30 A.M.

Rooms 230—257

R. G. CONNELLY, *Chairman**General Symposium on Extension Methods*

- E12—Display of extension teaching ideas. E. C. Scheidenhelm, Michigan State College, Chairman.  
Michigan—South Dakota—Nebraska—Iowa—Missouri—Wisconsin—Kansas—Texas—West Virginia—South Carolina—Alabama—Tennessee—Indiana.

*Panel Discussion on Extension Methods*

Leader: E. C. Scheidenhelm

Panel Members: Regan—Hayes—Johnson—Flack

Thursday, June 27, 1:00-4:00 P.M.

Room 363

R. G. CONNELLY, *Chairman*

- E13—Report of type classification committee. Jas. W. Linn, Kansas State College, Chairman.
- E14—Clinics for Dairy Herd Improvement Association fieldmen. A. J. Cramer, University of Wisconsin.
- E15—4H Dairy programs—Requirements and recommendations. Report of Calf Club Committee. H. A. Willman, Cornell University, Chairman.
- E16—Report of Quality Committee. H. R. Searles, University of Minnesota, Chairman.
- E17—An extension program in quality. J. M. Jensen, Michigan State College.

MANUFACTURING SECTION

Tuesday, June 25, 1:30-4:00 P.M.

Room 340

F. H. HERZER, *Chairman*

*Ice Cream*

- M1—The relation of acidity and total solids contents per gallon to the physical and chemical properties of high serum solids ice cream. C. W. Decker and W. C. Hall, Missouri Agricultural Experiment Station.
- M2—Characteristics of base exchange treated skim milk powder in ice cream. J. H. Erb, R. B. Hornberger and J. D. Bowers, Ohio State University.
- M3—Fresh and frozen plain, superheated and sweetened condensed skim milk for ice cream. L. K. Crowe, Darrell D. Deane, Harry H. Winn, University of Nebraska.
- M4—Replacing cane sugar with variable increments of dextrose sugars and the effect upon the physical and chemical properties of ice cream at different serving temperatures. R. J. Cooley, W. H. E. Reid and W. C. Hall, Missouri Agricultural Experiment Station.
- M5—Use of high conversion corn syrup in the manufacture of ice cream and ices. George J. Edman and P. H. Tracy, University of Illinois.
- M6—Corn sugar and sirups for frozen desserts. A. C. Dahlberg and E. S. Penczek, New York Agricultural Experiment Station.
- M7—Factors affecting the viscosity of ice cream mixes containing sodium-phospho-alginate. John H. Hetrick and J. H. Erb, Ohio State University.
- M8—Influence of drawing temperature as a factor affecting the stabilizing action of gelatin and the body and texture of batch and continuous frozen ice cream. R. E. Heyl and P. H. Tracy, University of Illinois.
- M9—The application of motion pictures as a medium in showing the influence of several factors upon the stability and meltdown properties of ice cream. W. S. Arbuckle, C. W. Decker and R. J. Cooley, Missouri Agricultural Experiment Station.

M10—A study of the coliform group in ice cream. H. J. Fournelle and H. Macy, University of Minnesota.

M11—Prevention of oxidized flavor in frozen cream by homogenization and high temperature pasteurization. G. C. McFarland and L. H. Burgwald, Ohio State University.

Wednesday, June 26, 9-11:30 A.M.

Room 340

*F. H. HERZER, Chairman  
Market Milk*

M12—A survey of the objectionable feed flavors in milk throughout the North American Continent. P. A. Downs, University of Nebraska.

M13—Interrelation of certain metals and metallic ions and the development of oxidized flavor in milk. O. F. Garrett, New Jersey Agricultural Experiment Station.

M14—A comparison of the effects of seven different types of roughages on the color and flavor of milk. O. F. Garrett, R. B. Arnold and G. H. Hartman, New Jersey Agricultural Experiment Station.

M15—Recent studies on oxidized flavor in milk. W. J. Corbett and P. H. Tracy, University of Illinois.

M16—Milk flavor study. H. B. Henderson, Thos. B. Harrison, and C. E. Wylie, University of Tennessee.

M17—The relationship of quality of hay to the development of oxidized flavor in milk. W. Carson Brown, A. H. VanLandingham and Chas. E. Weakley, Jr., West Virginia Agricultural Experiment Station.

M18—The effect of feeding cod-liver oil on the goaty and oxidized flavors, and vitamin C in milk. E. S. Guthrie, Cornell University.

M19—Resistance of thermoduric bacteria to chlorine disinfection. A. C. Maack and M. J. Prucha, University of Illinois.

M20—Is the standard plate count a proper yardstick of quality? M. E. Parker, Beatrice Creamery Company.

M21—Control of sediment in homogenized milk. A. J. Hahn and P. H. Tracy, University of Illinois.

Wednesday, June 26, 1:30-4:00 P.M.

Room 340

*F. H. HERZER, Chairman  
Market Milk and Butter*

M22—A study of the effect of added iodine and hydrogen peroxide to milk on the enzymes. Myer Glickstein, W. S. Mueller and J. H. Frandsen, Massachusetts State College.

M23—A study of the time-temperature relationships in the pasteurization of milk as regards creaming, phosphatase and bacterial destruction. R. F. Holland, and A. C. Dahlberg, New York Agricultural Experiment Station.

- M24—The relationship of changes in the chemical composition of milk to the development of mastitis. A. H. VanLandingham, Chas. E. Weakley, Jr., and E. N. Moore, West Virginia Agricultural Experiment Station.
- M25—The determination of copper in butter. W. F. Epple and B. E. Horrall, Purdue University.
- M26—The uniformity of butter composition as related to type of churn. S. L. Tuckey and P. H. Tracy, University of Illinois.
- M27—Changes in the bacterial flora of butter. C. A. Wilson and M. J. Prucha, University of Illinois.
- M28—Some preliminary observations on the effectiveness of propionates as mold inhibitors on dairy products. J. D. Ingle, Swift & Company.
- M29—Propionic acid and its calcium and sodium salts as inhibitors of mold growth. J. C. Olson and H. Macy, University of Minnesota.
- M30—Some of the factors affecting the phosphatase values of butter. W. H. Brown, Purdue University.
- M31—Effect of salt on the keeping quality of cream. W. J. Caulfield, F. E. Nelson, and W. H. Martin, Kansas Agricultural Experiment Station.

Thursday, June 27, 9-11:30 A.M.

Room 340

F. H. HERZER, *Chairman*  
*Cheese*

- M32—The chemical and bacteriological changes in brick cheese during manufacture. J. C. Garey, E. M. Foster and W. C. Frazier, University of Wisconsin.
- M33—The control of abnormal bacterial fermentations in the manufacture of Swiss cheese. Lloyd A. Burkey, Morrison Rogosa and Robert R. Farrar, Bureau of Dairy Industry, U. S. Dept. of Agriculture.
- M34—The effect of heat-treatment of milk on the activity of Swiss cheese starters. M. E. Tyler and H. H. Weiser, Ohio State University.
- M35—Standardization of fat in Swiss cheese and the relationship of fat to quality. George P. Sanders, Robert R. Farrar, Fred Feutz, and Robert E. Hardell, Bureau of Dairy Industry, U. S. Department of Agriculture.
- M36—Improving the quality of Swiss cheese through applied research and technical control. Robert R. Farrar, Bureau of Dairy Industry, U. S. Dept. of Agriculture.
- M37—Relation of salt content to bitter flavor development in cheddar cheese. S. L. Tuckey and H. A. Ruehe, University of Illinois.
- M38—More accurate determinations of volatile fatty acid and other changes as a means to study cheddar cheese curing. J. C. Marquardt and A. C. Dahlberg, New York Agricultural Experiment Station.
- M39—Effect of lipolytic enzymes on the ripening of cheddar cheese. C. B. Lane and B. W. Hammer, Iowa Agricultural Experiment Station.

- M40—The purification of rennin. C. L. Hankinson and L. S. Palmer, University of Minnesota.
- M41—The effect of standardizing the acidity on the methods and physical and chemical properties of cottage cheese and cultured buttermilk. L. E. Mull and W. H. E. Reid, Missouri Agricultural Experiment Station.
- M42—The use of homogenized milk in the manufacture of cottage cheese. D. W. Glover and L. H. Burgwald, Ohio State University.
- M43—The effect of temperature upon score value and serving properties of cheese. W. S. Arbuckle, J. E. Edmondson, and L. E. Mull, Missouri Agricultural Experiment Station.

Thursday, June 27, 1-4 P.M.  
Room 340

F. H. HERZER, *Chairman*

*By-Products, Bacteriology, Testing*

- M44—Economic barriers affecting the dairy industry. H. A. Ruehe, University of Illinois.
- M45—The effect of cocoa upon the digestibility of milk proteins. L. D. Lipman and W. S. Mueller, Massachusetts State College.
- M46—The acid hydrolysis of lactose and the preparation of hydrolyzed lactose sirup. G. A. Ramsdell and B. H. Webb, Bureau of Dairy Industry, U. S. Dept. of Agriculture.
- M47—Some properties of different combinations of whey and other materials which dry satisfactorily on the atmospheric drum drier. E. L. Jack and A. J. Wasson, University of California.
- M48—A more precise method for estimating fat in the Babcock Test. E. O. Herreid, Vermont Agricultural Experiment Station.
- M49—The effect of specific gravity and coefficient of expansion of butterfat on the accuracy of the Babcock Test. R. Jenness, Vermont Agricultural Experiment Station.
- M50—Observations on the distribution of *Pseudomonas fragi*. H. B. Morrison and B. W. Hammer, Kentucky and Iowa Agricultural Experiment Stations.
- M51—The serological integrity of *Streptococcus lactis*. J. M. Sherman, Karl L. Smiley, and Charles F. Niven, Jr., Cornell University.

PRODUCTION SECTION

Tuesday, June 25, 1:30-4:00 P.M.  
Room 350

*Joint Session with Extension Section*

A. H. KUHLMAN, *Presiding*

- E1—The nation-wide D.H.I.A. proved sire program. J. F. Kendrick, Bureau of Dairy Industry, U. S. Dept. of Agriculture.
- E2—The importance of selective registration to the dairy industry. Lynn Copeland, The American Jersey Cattle Club.

*Symposium on Artificial Insemination*

Discussion Leader : E. J. PERRY, *Chairman*, Better Sire Committee,  
New Jersey

- P1—Vitamin C for sterile and partially sterile sires. Paul H. Phillips and Henry A. Lardy, University of Wisconsin.
- P2—The storage of bull spermatozoa. H. A. Herman and Eric W. Swanson, University of Missouri.
- P3—Some observations on the morphological variations in the spermatozoa of dairy bulls. Eric W. Swanson and H. A. Herman, University of Missouri.
- P4—Fecundity and certain other characteristics of fresh and stored bovine semen. H. P. Davis, G. W. Trimberger, Gravers K. L. Underbjerg, University of Nebraska.

*Discussion Panel*

Phillips—Herman—Davis—Bartlett—Perry

Wednesday, June 26, 8:30-11:45 A.M.

A. H. KUHLMAN, *Chairman*  
Room 350  
*Milk Secretion*

- P5—Outlines and subject matter in teaching dairy husbandry courses. E. N. Hansen, Iowa State College.
- P6—An assay method for Thyrolactin. W. W. Heathman and C. W. Turner, Missouri Agricultural Experiment Station.
- P7—Thyrolactin, a new source of thyroxine for dairy cattle. C. W. Turner, Missouri Agricultural Experiment Station.
- P8—The effect of thyroxine injections on the physiological processes of dairy cattle. Victor Hurst, R. P. Reece and J. W. Bartlett, New Jersey Agricultural Experiment Station.
- P9—The ejection of milk from the mammary gland. Fordyce Ely and W. E. Petersen, Kentucky and Minnesota Agricultural Experiment Stations.
- P10—Effect of post-hypophyseal extract on lactation in hypophysectomized post-gravid rats. Eliseo T. Gomez, Bureau of Dairy Industry, U. S. Dept. of Agriculture.
- P11—The fat metabolism of the mammary gland of the cow. J. C. Shaw and W. E. Petersen, University of Minnesota.
- P12—Some factors influencing the completeness of milking. Kenneth Miller and W. E. Petersen, University of Minnesota.
- P13—The effect of dinitrophenol administration on milk and milk fat. G. C. Graf, L. M. Ludwick and W. E. Petersen, University of Minnesota.
- P14—The pH of the bovine mammary gland. Philip L. Kelly, Arkansas Agricultural Experiment Station.
- P15—The hormone control of mammary duct growth. A. A. Lewis, Missouri Agricultural Experiment Station.

- P16—The mammogenic lobule-alveolar factor of the anterior pituitary.  
John P. Mixner, Missouri Agricultural Experiment Station.
- P17—The effect of nembutal anesthesia on the rate of milk secretion, the respiratory quotient, and uptake of milk precursors by the lactating mammary gland. E. P. Reineke, Missouri Agricultural Experiment Station.
- P18—A modification of the Allen blood fat procedure. J. C. Shaw, University of Connecticut.

Wednesday, June 26, 1:30-5:00 P.M.

Room 350

**A. H. KUHLMAN, Chairman**

*Breeding, Disease, Calf Feeding*

- P19—A study of some methods for the prediction of butterfat percentage in herds of Ayrshire cattle. G. A. Bowling and D. N. Putnam, West Virginia Agricultural Experiment Station.
- P20—The use of cellular antigens in the blood of cattle for determining parentage. L. C. Ferguson and M. R. Irwin, University of Wisconsin.
- P21—Effects of inbreeding in dairy cattle. G. E. Dickerson, Wisconsin Agricultural Experiment Station.
- P22—Results of twenty years work on proving bulls at the Huntley, Montana, field station. R. R. Graves, J. R. Dawson, and D. V. Kopland, Bureau of Dairy Industry, U. S. Department of Agriculture.
- P23—Average useful life-span, and causes of losses of dairy bulls. R. B. Becker and P. T. Dix Arnold, Florida Agricultural Experiment Station.
- P24—The inheritance of the solids-not-fat percentage in dairy cattle. H. C. Moore and K. S. Morrow, New Hampshire Experiment Station.
- P25—Some factors affecting breeding efficiency in dairy cattle. R. E. Erb, J. W. Wilbur and J. H. Hilton, Purdue University.
- P26—Early recognition of the freemartin condition in heifers twin-born with bulls. W. W. Swett, C. A. Matthews and R. R. Graves, Bureau of Dairy Industry, U. S. Dept. of Agriculture.
- P27—Some factors relating to bloat in cattle. Dwight Espe and C. Y. Cannon, Iowa State College.
- P28—Extreme rarity of cancerous growths in the cow's udder. W. W. Swett, C. A. Matthews and R. R. Graves, Bureau of Dairy Industry, U. S. Dept. of Agriculture.
- P29—Heavy corn feeding as a contributory factor to the development of mastitis. Earl N. Moore and H. O. Henderson, West Virginia Agricultural Experiment Station.
- P30—Short-wave diathermy treatment of bovine mastitis. C. W. McIntyre, A. C. Ragsdale, and E. R. Garrison, Missouri Agricultural Experiment Station.
- P31—Purified diet studies with calves. P. E. Johnson, J. K. Loosli, and L. A. Maynard, Cornell University.

- P32—Changes in pH and in bacterial count of milks sham fed to a dairy calf. George H. Wise, G. W. Anderson and J. C. Jones, South Carolina Agricultural Experiment Station.

Thursday, June 27, 8:30-12:00 A.M.

Room 350

A. H. KUHLMAN, *Chairman*

*Nutrition*

- P33—Studies with barn air-cured alfalfa hay. C. E. Wylie, S. A. Hinton, and J. A. Schaller, University of Tennessee and Tennessee Valley Authority.
- P34—Dried grapefruit pulp for milk production. P. T. Dix Arnold, R. B. Becker and W. M. Neal, Florida Agricultural Experiment Station.
- P35—The value of the qualitative color test in the study of ketosis. C. W. Duncan and C. F. Huffman, Michigan Agricultural Experiment Station.
- P36—Blood sugar and carbon dioxide combining power of plasma in relation to ketosis in dairy cattle. J. F. Sykes, C. W. Duncan and C. F. Huffman, Michigan State College.
- P37—The relationship of fat content in the dairy ration to milk and butterfat production. C. F. Monroe and W. E. Krauss, Ohio Agricultural Experiment Station.
- P38—Alfalfa hay cut at three stages of maturity; its yield, chemical composition and feeding value for milk production. J. R. Dawson, D. V. Kopland, and R. R. Graves, Bureau of Dairy Industry, U. S. Dept. of Agriculture.
- P39—Cystine as a possible deficiency in a ration of alfalfa hay for milk production. C. F. Huffman and C. W. Duncan, Michigan Agricultural Experiment Station.
- P40—The feeding value of rye stillage for dairy cows. K. L. Turk and M. H. Berry, Maryland Agricultural Experiment Station.
- P41—Fermentation studies on alfalfa silage prepared by the phosphoric acid and molasses methods. H. D. McAuliffe, R. W. Stone and S. I. Bechdel, The Pennsylvania State College.
- P42—The losses resulting from the ensiling of legumes and grasses with varying amounts of phosphoric acid. O. L. Lepard and E. S. Savage, Cornell University.
- P43—Effect of depth of corn in the silo on weight of corn silage. Joseph B. Shepherd, Bureau of Dairy Industry, U. S. Dept. of Agriculture.
- P44—Broomcorn silage for dairy cattle. K. E. Harshbarger and W. B. Nevens, University of Illinois.
- P45—Comparison of *Lespedeza Sericea* silage, alfalfa silage, and corn silage for dairy cows. S. A. Hinton and C. E. Wylie, University of Tennessee.
- P46—Composition and nutrient value of sugarcane as fresh forage, shocked fodder and silage. W. M. Neal, Florida Agricultural Experiment Station.

Thursday, June 27, 1:00-4:00 P.M.  
Room 350

A. H. KUHLMAN, *Chairman*

*Minerals and Vitamins*

- P47—Is timothy hay adequate in calcium for optimum growth of dairy heifers? H. T. Converse, Edward A. Kane, and Edward B. Meigs, Bureau of Dairy Industry, U. S. Dept. of Agriculture.
- P48—The effect of rations deficient in phosphorus and protein on ovulation, estrus and reproduction in dairy heifers. L. S. Palmer, T. W. Gullickson, W. L. Boyd, C. P. Fitch and J. W. Nelson, University of Minnesota.
- P49—The effect of avitaminosis-A upon vitamin C in the bovine. W. A. King, P. H. Phillips, M. E. Nesbit, I. W. Rupel and G. Bohstedt, University of Wisconsin.
- P50—Vitamin C in the nutrition of dairy cattle. G. C. Wallis, South Dakota Agricultural Experiment Station.
- P51—Blood-plasma magnesium in relation to the vitamin D deficiency of mature dairy cattle. G. C. Wallis, South Dakota Agricultural Experiment Station.
- P52—Vitamin E potency of certain feedstuffs. L. S. Palmer, J. W. Nelson and T. W. Gullickson (with the assistance of B. B. Migicovsky and W. W. Kielley), University of Minnesota.
- P53—Carotene content of corn silage. Edward A. Kane, Herbert G. Wiseman, Leo A. Shinn, and C. A. Cary, Bureau of Dairy Industry, U. S. Dept. of Agriculture.
- P54—Changes in the amounts of carotene and vitamin A and in the composition of milk fat in artificially induced mastitis. P. G. Miller, E. J. Lease and G. W. Anderson, South Carolina Agricultural Experiment Station.
- P55—The effects of vitamin A deficiency on the young male bovine. T. S. Sutton, W. E. Krauss and S. L. Hansard, Ohio Agricultural Experiment Station and Ohio State University.
- P56—Cerebrospinal fluid pressure and vitamin A deficiency. L. A. Moore and J. F. Sykes, Michigan Agricultural Experiment Station.
- P57—The effect of carotene consumption on the milk yield of Jersey cows. O. C. Copeland, Texas Agricultural Experiment Station.
- P58—The vitamin A requirements of dairy cows for the production of butter of high vitamin A value. II. Relative efficiency of carotene (dehydrated alfalfa hay) and vitamin A. J. W. Wilbur, J. H. Hilton and S. M. Hauge, Purdue University.



## ABSTRACTS OF PAPERS

### MANUFACTURING SECTION

#### **M1. The Relation of Acidity and Total Solids Contents per Gallon to the Physical and Chemical Properties of High Serum Solids Ice Cream.\* C. W. DECKER AND W. C. HALL, Missouri Agricultural Experiment Station.**

This investigation included a study of the relation of the acidity, total solids per gallon, and variable increments of serum solids in the mix to the flavor, body, crystalline structure, dipping qualities and chemical properties of high serum solids ice cream.

Consumer preference of ice creams containing 13.50 per cent and 15.00 per cent serum solids content with the acidity adjusted to 0.24, 0.18, 0.12 and 0.08 per cent showed that the flavor of the low acid ice cream was preferred at the lower temperatures and the flavor of the higher acidity ice cream was preferred at the higher temperatures. As the per cent acidity decreased from 0.24 to 0.08 per cent, the crystal size decreased to a certain extent and then slightly increased again at the lowest acidity.

At the higher acidity and lower pH, the weight per "disher" was greatest and the stability was progressively greater as the acidity decreased in the 13.50 per cent serum solids ice cream. However, this was not as noticeable in the 15.00 per cent serum solids ice cream. There appeared to be a relationship between the acidity and pH and the viscosity and freezing properties of the mix.

The weight per gallon of ice cream was varied from 1.65 to 2.06 pounds. Consumer observations indicate that the flavor and body was more desirable in the medium weight per gallon ice creams, and dipping studies show that as the total solids per gallon decreased, the "disher" size increased at the lower temperatures. However, the reverse was true at higher dipping temperatures, and as the weight of total solids per gallon increased the stability decreased.

The results of this study indicate that the acidity, weight of total solids per gallon and per cent serum solids content has a pronounced effect upon the physical and chemical properties of the finished ice cream.

#### **M2. Characteristics of Base Exchange Treated Skimmilk Powder in Ice Cream. J. H. ERB, R. B. HORNBERGER AND J. D. BOWERS, Ohio State University.**

Base-exchange treated spray-process skimmilk powder was used in varying amounts to supply serum solids in ice cream mixes, and these were

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compared with mixes of the same composition containing serum solids from regular spray-process skimmilk powder and skimmilk. In producing the special milk powder the skimmilk was not acidified before being sent through the zeolite so that the final reaction of the dried milk was pH 7.16. The titratable acidity on the reconstituted basis was .06 per cent. In later work it was found that skimmilk, acidified so that the final pH of the powder was 6.5 before being sent through the zeolite, responded similarly to the unacidified.

The most significant difference in the use of the base-exchange powder in ice cream mixes was the more rapid whipping in batch freezers of mixes containing this product. The rapidity of whipping varied with the amount of base exchange powder used, but a quantity as small as 10 per cent of the total amount of serum solids content showed more rapid whipping. The mixes containing the zeolite treated solids were of lower viscosity than the control mixes.

Ice cream containing base exchange treated powder melted slightly more rapidly than ice cream of the same composition containing regular powder. The rapidity of melt down was in proportion to the amount of base exchange product used.

Figures will be given showing the distribution of minerals in the zeolite treated milk.

### M3. Fresh and Frozen Plain, Superheated and Sweetened Condensed Skimmilk for Ice Cream.\* L. K. CROWE, DARRELL D. DEANE AND HARRY H. WINN, University of Nebraska.

A study was made of some characteristics of a commercial milk supply in relation to the manufacture and storage of condensed skimmilk for use in ice cream as well as the suitability of stored frozen condensed milk of three types as a source of added serum solids in ice cream.

Chemical analysis of the whole milk supply did not reveal a significant correlation between the characteristics studied and the degree of stability of the protein fraction of the plain condensed skimmilk manufactured from it and held at 0° F. for four weeks.

Plain and sweetened condensed skimmilks after being held frozen for periods up to three months were satisfactory sources of serum solids for ice cream. The protein in superheated condensed skimmilk showed precipitation after one month's storage at 0° F. and precipitation increased rapidly with longer storage periods.

There were no significant differences in protein stability, pH, titratable acidity and viscosity of ice cream mixes made with the three types of fresh and stored frozen condensed skimmilk.

\* The data presented in this paper are from a study made by the junior authors under the supervision of the senior author in partial fulfillment of the work required for the degree of Master of Science.

Ice cream mixes made with fresh plain condensed skimmilk whipped to 100 per cent overrun slightly faster and to a slightly higher maximum overrun than when fresh superheated or fresh sweetened condensed milk was used. Superheated and sweetened condensed skimmilk mixes were equal in time to reach 100 per cent overrun but the former did not reach as high maximum overrun. Freezing and storing condensed skimmilk for 3 months at 0° F. reduced the time to reach 100 per cent overrun in the mixes in which it was used.

There was no appreciable difference in flavor score of the mixes containing either of the three types of condensed milk when used fresh or after storage.

Ice cream made with fresh or stored frozen superheated condensed skimmilk was more resistant to melting and gave less foam on melting than ice cream made with plain and sweetened condensed skimmilk.

**M4. Replacing Cane Sugar with Variable Increments of Dextrose Sugars and the Effect upon the Physical and Chemical Properties of Ice Cream at Different Serving Temperatures.\* R. J. COOLEY, W. H. E. REID AND W. C. HALL, Missouri Agricultural Experiment Station.**

The object of this investigation was to obtain technical data relating to replacing cane sugar with variable increments of dextrose sugar and its effect upon the ice cream mixes and the resulting ice cream.

The partial replacement of cane sugar in ice cream by corn sugar seems to be a desirable manufacturing procedure when the approximate replacement is 25 per cent. The replacement in this investigation ranged from 16.7 per cent to 44.4 per cent in mixes varying in total sugar content from 12 to 18 percent.

Motion and still pictures were used in studying the stability and melt-down qualities. Microphotographs and macrophotographs were used to show the crystalline and air cell structure of the different ice creams. Regardless of the amount of cane sugar replaced by dextrose or cerelose up to 18 per cent total sugar and 44.4 per cent replacement, there appeared to be no marked variation between all sucrose mixes.

Consumer preference studies on fifteen different mixes were conducted in cooperation with over 400 individual men and women. Fourteen classes of four to five samples of ice cream per class were given relative placings and specific criticisms by these representative consumer judges. Tabulations have been made on 3,465 samples of ice cream for flavor, body, and texture. The serving temperatures used were 4, 8, 12 and 16° F. respectively.

It appeared that a replacement of 25 to 35 per cent dextrose or cerelose

\* Missouri Agr. Experiment Station, Journal Series No. 628.

for an equal weight of sucrose in ice creams was acceptable and, in some instances, preferred by the average consumer of ice cream.

#### M5. Use of High Conversion Corn Sirup in the Manufacture of Ice Cream and Ices. GEORGE J. EDMAN AND P. H. TRACY, University of Illinois.

The introduction of a new type of corn sirup (known commercially as Sweetose) with an increased dextrose equivalent and reduced dextrin content as compared with the regular type of corn sirup, has made it desirable to determine the possibilities of the use of this product as a sweetening agent in the manufacture of ice cream, water ices, and sherbets.

Studies have been made of the effect of the use of the sirup upon the physical properties of the mix and ice cream. In the same way the merits of the sirup in ices have been determined.

It has been found possible to replace as much as 33½ per cent of the sucrose in ice cream and 50 per cent of the sucrose in water ices and sherbets without any undesirable effects. The sweetening value assigned to the high conversion sirup was two-third that of sucrose. The superior body effect resulting from the use of the sirup was thought to be due to the higher total solids content resulting as well as the effect of the dextrins and the slightly greater depressing effect upon the freezing point that the sirup has as compared with sucrose.

No deleterious flavor effects were observed, and in some cases the added flavors were intensified by the sirup.

While the corn sirup lowered the freezing point of the mix (one-third replacement) as compared with the sucrose control mix, the effect was no greater than in the case of a mix containing dextrose (one-fifth replacement).

Corn sirup was found to be soluble in water solutions frozen and stored at -15° F. This property of the sirup was found to be a decided advantage in preventing surface crustation in water ices.

#### M6. Corn Sugar and Sirups for Frozen Desserts. A. C. DAHLBERG AND E. S. PENCZEK, New York Agricultural Experiment Station.

In this study three types of corn sweeteners were compared with sucrose, namely a new type of liquid corn sirup, d. e. 64.5, a new dry corn sirup, d. e. 42.5 and corn sugar. These sweeteners were compared in water solutions, in ice cream, and in ices.

There are differences in the flavor, pH, relative sweetness, and freezing point depression of these corn sweeteners. Only one of the three products had a "sirup flavor" in concentrations used in ice cream. The pH values of both sirups were below that of the ice cream mix. Both corn

sirups, on a dry basis, depressed the freezing point of water about the same as sucrose whereas corn sugar showed a greater depression.

In the ice cream mix it was found that 25 per cent of the sucrose could be replaced with corn sweeteners as larger amounts produced soft ice cream. All of the corn sweeteners reduced the freezing points of the mixes more than sucrose when used in quantities sufficient to give comparable sweetness. However, the dry corn sirup was used in reduced quantities and it produced a mix with a high freezing point and an ice cream slightly firmer than sucrose ice cream.

The use of corn sweeteners had no material effect upon the acidity, pH, viscosity, surface tension, fat clumping and whipping properties of the mixes.

The various corn sweeteners varied somewhat in their effect upon the flavor of ice cream. The ice cream with liquid corn sirup possessed a slightly fresher, fuller flavor than sucrose ice cream. The dry corn sirup ice cream was a trifle flat in flavor due chiefly to too low a sweetening value indicating clearly the need for additional sugar. In most instances the liquid corn sirup ice cream possessed slightly superior keeping qualities. The body and texture of the ice cream was improved slightly by both sirups.

The hardness of ice cream was decreased and the rate of melting increased most for corn sugar and to a lesser degree for the corn sirups. However, in the concentrations used this effect was commercially insignificant.

The development of sandiness was not greatly affected by the sugars yet there was a tendency for the dry corn sirup to slightly retard its development.

In all cases the use of corn sweeteners eliminated the development of sucrose crystallization in ices. These corn products are essential in sherbets and ices to manufacture a commercial product of satisfactory keeping quality.

#### M7. Factors Affecting the Viscosity of Ice Cream Mixes Containing Sodium-Phospho-Alginate. JOHN H. HETRICK AND J. H. ERB, Ohio State University.

The ice cream stabilizer, sodium-phospho-alginate, commercially known as Dariloid, increases the viscosity of ice cream mix appreciably when used in the amount necessary for proper stabilization. The quantity used in ice cream ranges from .25 per cent to .30 per cent. The high viscosity of mixes containing this algin is one of the main factors limiting its use.

A number of factors have been studied which have an effect on the viscosity. When a small amount of di-sodium phosphate or sodium citrate

was added to the mix before incorporation of .25 per cent algin the viscosity of the mix was increased over the control containing no added salt. The pH of the mix previous to incorporating the algin was very important from the standpoint of viscosity. In a range from pH 6.2 to pH 7.2 the viscosity increased in proportion to the increase in pH.

Pasteurization temperatures have an influence on the viscosity of mixes stabilized with sodium-phospho-alginate just opposite to those stabilized with gelatin. Temperatures were studied from 160° F. to 180° F. The higher temperatures in the case of the algin mixes produced a greater immediate viscosity and this viscosity was retained on aging. The ice cream made from mixes pasteurized at 180° F. showed greater melting resistance and slightly better body and texture than mixes containing the same amount of algin but pasteurized at 160° F. It was also found that the longer the time the algin mixes were held at the pasteurizing temperature the greater was the final viscosity of the mix.

**M8. Influence of Drawing Temperature as a Factor Affecting the Stabilizing Action of Gelatin and the Body and Texture of Batch and Continuous Frozen Ice Cream.** R. E. HEYL AND P. H. TRACY, University of Illinois.

In a series of experiments dealing with the stabilizing power of gelatin in ice cream, it was observed that identical mixes when frozen in a counter freezer, a horizontal batch freezer, and in a continuous type of freezer showed little correlation in body score of the resulting ice creams.

When drawing temperatures were compared, the ice creams with the lowest drawing temperatures were found to have the highest body and texture scores, and a study was undertaken to determine the relation of drawing temperatures to the body and texture of ice cream.

Ice cream mixes (12 per cent fat, 11 per cent serum solids, 15 per cent sugar) containing 0.28 per cent, 0.30 per cent, and 0.35 per cent of 225 Bloom porkskin gelatin (pH 4.5) were prepared and frozen in a continuous freezer to 24.4° F., 23.8° F., and 22.1° F. at 100 per cent overrun when drawn. When these samples were judged, it was found that the body and texture scores varied inversely with the drawing temperatures. The ice cream drawn at 22.1° F. had a smoother body than any samples drawn at higher temperatures.

Since the freezing point of an ice cream mix and its whipping ability directly influence the temperature at which it can be drawn from the freezer, the study was extended to include ice cream frozen in a counter freezer, and in a horizontal 40-quart batch freezer. Ice cream frozen in these types of freezers could not be drawn at 100 per cent overrun at as low temperatures as in the continuous type freezer.

In all cases, ice creams that were drawn at the same temperatures regardless of the type of freezer, compared favorably with each other from the standpoint of body, when analyzed by organoleptic tests.

Overfreezing on the continuous freezer or drawing ice creams at temperatures too low, when the quantity of gelatin present was sufficient for higher drawing temperatures, tended to produce ice creams with sticky or gummy bodies.

The results suggest that due consideration should be given to possible variations in drawing temperature of the ice cream in attempting to arrive at the optimum amount of stabilizer that should be used in the mix.

**M9. The Application of Motion Pictures as a Medium in Showing the Influence of Several Factors upon the Stability and Meltdown Properties of Ice Cream.\*** W. S. ARBUCKLE, C. W. DECKER AND R. J. COOLEY, Missouri Agricultural Experiment Station.

Studies have been made by the use of motion picture photography in showing the relation of several factors in the composition and manufacturing procedure upon the stability of vanilla ice cream.

The investigation includes the effect of variable acidity in medium and high serum solids content mixes, the effect of overrun or weight per gallon of ice cream, the different sources of serum solids and of replacing variable increments of sucrose with dextrose and cerelose in medium and low fat content mixes upon the stability and melt down properties.

The pictures illustrate the effectiveness of motion pictures in presenting complete detailed information of educational and investigational value.

**M10. A Study of the Coliform Group in Ice Cream.** H. J. FOURNELLE AND H. MACY, University of Minnesota.

A study is being made of the numbers and types of the coliform group in ice cream. Factory-packaged samples obtained from ice cream manufacturers have been vanilla, chocolate, strawberry, sherbet (or ice), and a chocolate-covered confection known as "cheerio." Scoop, or dipper, samples have been obtained from drug store fountains, confectioneries, ice cream shops, etc.

Brilliant green-lactose-bile broth has been used for presumptive tests. An estimation of the numbers is made by using the dilution method of Halvorson and Ziegler (*Jour. Baet.*, 25: 101-121, 1933). For factory-packed ice cream, six dilutions are made with 10 tubes inoculated in each dilution. The lowest dilution, consisting of 10 ml. of the sample, is inoculated into 100 ml. of brilliant green-lactose-bile medium. The other dilutions, 1 ml. to 0.0001 ml., inclusive, are inoculated into 10 ml. quantities of the medium. After 48 hours incubation at 37° C. all tubes are ex-

\* Paper No. 629 in the Missouri Agricultural Experiment Station Journal Series.

amined for gas production. Halvorson and Ziegler's tables giving the most probable number of bacteria per ml. are used for interpreting data. The range of dilutions of inocula of scoop samples is between 1 ml. and 0.000,001 ml., inclusive, where 10 tubes are used for each dilution. Direct plate counts are also made on violet red-bile agar.

A comparison is being made between buffered and unbuffered brilliant green-lactose-bile broth to determine which medium is the more suitable for sherbets and ices. Five tubes each of buffered and unbuffered medium are used in each dilution.

Eosin-methylene blue agar plates are streaked from tubes of brilliant green-lactose broth of the lowest and highest dilutions showing gas after 24 and 48 hours. Representative typical and atypical coliform colonies and non-coliform colonies are picked and transferred to nutrient agar slants.

All cultures are purified before planting into differential media. For purification light suspensions are made in sterile distilled water from agar slants and inocula from this are streaked on eosin-methylene blue agar. After incubation, representative colonies of different types are picked and transferred to nutrient agar slants. This process is repeated so that three E. M. B. plates are streaked and colonies transferred to three agar slants.

Gram and flagella stains and determination of motility are made from nutrient agar slant cultures that have incubated 18-22 hours at 37° C. The method of study of bacterial flagellation recommended by Conn and Wolfe (*Jour. Bact.* **36**: 517-520. 1938) is being used.

The following tests and reactions are used for identification of the types isolated: fermentation of lactose, dextrose, sucrose, salicin, dulcitol, and glycerol; formation of indol from 1 per cent tryptone broth; utilization of citrate as sole source of carbon; methyl red and Voges-Proskauer reactions; reduction of nitrate; hydrogen sulphide production; gelatin liquefaction, and action on litmus milk.

Identification of cultures is made according to Bergey's Manual of Determinative Bacteriology, 5th Edition.

The probable numbers of the coliform types in the samples studied range from less than one per ten milliliters to 1160 per milliliter. The following types have been isolated, *Escherichia coli*, *E. coli* var. *acidilactici*, *E. coli* var. *neapolitana*, *E. freundii*, *Aerobacter aerogenes*, *Aerobacter cloacae*, and a variety of other Gram negative rods that were isolated from eosin-methylene blue plates.

#### M11. Prevention of Oxidized Flavor in Frozen Cream by Homogenization and High Temperature Pasteurization. G. C. MFARLAND AND L. H. BURGAWALD, Ohio State University.

Due to the lack of balance between production and utilization of milk over the yearly period, some means of storing surplus is necessary. One

method used is that of storing cream in the frozen state; however, considerable trouble from oxidized flavor is encountered.

Three trials were run using high temperature pasteurized cream; the cream being pasteurized at 172° F. for one minute and for five minutes. In one of these trials highly susceptible cream was used. The cream used was separated from milk which had been pasteurized at 145° F. for 30 minutes. Copper was added to the cream in the form of copper sulfate in amounts from 0.5 to 2.5 p.p.m. just before pasteurization at 172° F. Eight minutes were required to bring the temperature from 45° F. to 172° F. The cream was stored in waxed paper cartons at temperatures ranging between minus ten and zero degrees F.

Three homogenized trials were also run in which the copper was added after pasteurization at 145° F. for 30 minutes, but before homogenization at 2300 to 2500 pounds pressure. The temperature at time of homogenization was 130° F. Highly susceptible cream was also used in one of these trials. The cream was stored in waxed paper cartons the same as for the other cream.

In one trial, cream pasteurized at 172° F. for one minute and five minutes did not go oxidized after five months storage. In another trial, those samples contaminated with 2.0 and 2.5 p.p.m. copper and pasteurized at 172° F. for one minute showed a trace of oxidized flavor in four months. None was found in any of the samples pasteurized at 172° F. for five minutes. In the trial with susceptible cream, the copper contaminated samples pasteurized at 172° F. for one minute went oxidized in one and one-half months; however, the intensity was much less than that of the control samples. No oxidized flavor developed in the samples pasteurized at 172° F. for five minutes in this trial by the end of two months.

In all of the trials, the copper-contaminated control samples developed the flavor in one and one-half months or less.

Although an objectionable cooked flavor was present in the cream pasteurized at 172° F. at first, it diminished enough to be unobjectionable after one month's storage.

Results obtained seem to indicate that high temperature pasteurization at 172° F. for five minutes was sufficient to prevent the formation of oxidized flavor in frozen cream.

In one trial, the homogenized samples contaminated with 2.0 and 3.0 p.p.m. of copper developed the flavor in the sixth month. In another trial, none of the homogenized cream developed the flavor after four months, and in the trial with susceptible cream, none of the homogenized samples had gone oxidized after two months storage. All the copper-contaminated unhomogenized control developed the flavor in one and one-half months or less.

Homogenization seems to be effective in preventing the development of the oxidized flavor.

In a trial to note the effect of pancreatic enzyme as a preventive, copper-contaminated samples containing 2.0, 5.0, and 10.0 p.p.m. of pancreatic enzyme have been in storage two months with no development of oxidized flavor. All of the copper-contaminated controls have developed the flavor in one and one-half months or less.

**M12. A Survey of the Objectionable Feed Flavors in Milk Throughout the North American Continent.** P. A. DOWNS, University of Nebraska.

A survey of the North American continent by states and provinces has been made in an endeavor to ascertain the seriousness of feed and weed flavors in milk and milk products. The results indicate that it is a serious problem in the majority of the territories. Feed flavors are reported as objectionable in widely distributed areas while weeds are reported as being the cause of trouble more extensively in the Middle West and South. Silage contributes in a great many cases, followed by pasture such as rye and sweet clover with alfalfa hay being reported as a cause of flavor in many states. Onion probably is the most common source of trouble in the weed class followed by ragweed and bitter weed. French weed is reported as being a serious problem in the Great Plains area and the Canadian Middle West. In those states where the problem is serious many experimental projects are in progress. Rather a limited file of references are available in the various states, and circulars, bulletins, and printed material available vary with the importance of the problem in the particular state. The educational program in various states that are seriously affected covers the use of the newspaper, radio, extension service, group meetings, 4-H Club, and in some states vocational agriculture high schools.

From the results obtained it is apparent that the problem is serious in the North American continent as a whole, being spread from coast to coast and from Canada to the Gulf of Mexico in one form or another. It is believed that more attention should be given this problem and that greater effort should be made to impress the producer with the importance of feed management for the dairy herd.

**M13. Interrelation of Certain Metals and Metallic Ions and the Development of Oxidized Flavor in Milk.** O. F. GARRETT, New Jersey Agricultural Experiment Station.

While studying the accelerative action of cupric and ferrous ions on the development of oxidized flavor in milk, it was observed that in one case, where equal molar proportions of both ions were added to a sample of milk, the accelerative effect was not so great as that of either one of the ions when added alone. This led to further studies of the interaction of various other metals and their ions when placed in milk.

In these studies copper sulfate was added in various molar concentrations to milk immediately following which various molar concentrations of other metallic salts were added. The results are summarized as follows:

When divalent manganese was added in molar concentration equal or greater than copper the development of the oxidized flavor was either greatly retarded or completely inhibited for periods up to 96 hours.

In two samples of milk which spontaneously developed the oxidized flavor no such flavor appeared in the milk containing divalent manganese but no copper.

The addition of ferrous iron to samples of milk containing added copper greatly retarded but usually did not completely eliminate the development of the oxidized flavor.

The addition of divalent manganese to samples of milk containing ferrous iron either greatly retarded or completely inhibited the development of oxidized flavor up to 72 hours.

The addition of trivalent aluminum or of ferric iron to samples of milk which contained copper did not retard the development of the oxidized flavor.

When pieces of manganese metal were placed in milk containing various concentrations of copper sulfate or ferrous sulfate the development of the oxidized flavor was greatly retarded.

When strips of copper metal were placed in milk containing divalent manganese or pieces of manganese metal the development of the oxidized flavor was greatly retarded.

Divalent manganese added to milk containing copper after the development of the oxidized flavor had begun checked further development of the flavor.

The addition of divalent manganese to milk containing copper had no effect on the oxidation rate of reduced ascorbic acid nor on the magnitude of the oxidation-reduction potential.

**M14. A Comparison of the Effects of Seven Different Types of Roughages on the Color and Flavor of Milk.** O. F. GARRETT, R. B. ARNOLD AND G. H. HARTMAN, New Jersey Agricultural Experiment Station.

In the first experiment a comparison was made between silage made from immature alfalfa preserved with molasses and green spring pasture. The color of the milk showed most of its increase during the first 4 weeks of feeding the silage but the maximum level was not reached until approximately 10 weeks had passed. The maximum color of the milk produced on the alfalfa silage (6.3 lactochrometer units) was almost equal to that produced after 3 weeks on green pasture (6.4 lactochrometer units).

The flavor score of the milk produced on the alfalfa silage reached a maximum (above 22) in about 4 weeks and maintained this level until the cows were put on pasture when there was a slight but definite drop due to the appearance of "feed" flavors.

In a second experiment a comparison was made of the effects of feeding molasses grass silage, phosphoric acid grass silage and corn silage on the color and flavor of milk. Three groups of cows were fed continuously on the three types of roughages for a period of 18 weeks. The following average values were obtained at the beginning of the experiment; color, 5.2 lactochrometer units; fresh milk score, 20.8; milk score after 72 hours, 19.3; oxidation score after 72 hours with copper, 3.6. Similarly, the averages for the experimental period on the three roughages were: molasses silage 6.3, 22.1, 21.1 and 1.6; phosphoric acid silage 6.1, 21.9, 20.9 and 1.5; corn silage 5.2, 20.6, 19.4 and 3.4. The grass silage preserved by either method was definitely superior to corn silage for producing milk of high yellow color and good flavor stability but no significant differences occurred in the effects of the two grass silages.

In a third experiment the effects on color and flavor of milk produced on molasses grass silage, beet pulp, and molasses-impregnated citrus pulp were studied. One group of cows was fed in 4-week periods, respectively, beet pulp, citrus pulp and grass silage. A second group was fed, respectively, grass silage, citrus pulp and beet pulp.

The average color in lactochrometer units for the three roughages was as follows: beet pulp, 4.9; citrus pulp, 4.8; grass silage 5.6. Similarly, the average flavor scores for the fresh milk were: beet pulp, 20.5; citrus pulp, 20.6; grass silage, 21.7; flavor scores after 72 hours were: beet pulp, 19.4; citrus pulp, 19.5; grass silage, 20.9; oxidation scores after 72 hours with copper were: beet pulp, 4.1; citrus pulp, 3.8; grass silage, 2.3.

Beet pulp and citrus pulp, impregnated with molasses, appear to be inferior to grass silage in producing milk of high color and good flavor. No significant difference with respect to these two factors was noted between beet pulp and citrus pulp.

#### M15. Recent Studies on Oxidized Flavor in Milk. W. J. CORBETT AND P. H. TRACY, University of Illinois.

Various investigators have suggested that the degree of saturation of the milk fat was related to the oxidation of the fat and occurrence of oxidized flavor. The saturation of the fat was varied by feeding one group of 3 cows cocoanut oil and another group of 3 cows corn oil. The oils were fed for a period of 12 days, omitted for 10 days, and then the groups were reversed and again fed corn and cocoanut oil for a period of 12 days. The cocoanut oil lowered the iodine number approximately 4 per cent and the corn oil increased the iodine number approximately 15 per cent. Each group of

cows contained one animal that gave milk which developed the oxidized flavor "spontaneously," and two cows whose milk developed the oxidized flavor in the presence of copper, one cow's milk being more resistant to copper than the other. All samples of milk were pasteurized in glass immediately after milking and divided into several lots. Copper sulphate was added to some of the milk samples. Changing the degree of saturation of the fat had no effect on the development or occurrence of the oxidized flavors.

Studies of the anti-oxidative effect of tyrosine and the more soluble tyrosine esters have shown them to be very effective anti-oxidants in milk when added at the rate of .02 per cent-.03 per cent.

**M16. Milk Flavor Study.** H. B. HENDERSON, THOS. B. HARRISON AND C. E. WYLIE, University of Tennessee.

For two years investigations have been conducted at the Tennessee Station relative to the preservation and feeding of legume silage. This year a project has been conducted in conjunction with this work to determine what effect feeding various rations to dairy cows might have upon the flavor of milk. Data relative to the flavor score, flavor criticisms and susceptibility of the milk to the development of oxidized flavor have been obtained.

Four groups of cows were used in this project. Groups I, II, and III were fed rations comparing alfalfa, corn, and sericea silages. Group IV, which was composed of all the cows on official test in the University herd, received a much heavier ration than either of the other three groups.

A comparison of flavor scores of milk from individual cows over a period of several months during the winter of 1939-1940 would indicate that although the feed consumed by cows does have some effect upon the flavor of the milk, as is evidenced by the presence of a feed flavor in the milk, this effect varies considerably between cows. Marked variations were consistently noted in the flavor score of milk from individual cows receiving identical rations. It was also noted that individual cows in a particular group produced milk that varied considerably in flavor score from one scoring period to the next, and no one cow was found to produce milk having a consistently high or low score. From the data collected to date, it would appear that other factors may have at least as much effect upon the flavor of the milk as the feed the cows consume.

Samples of milk from individual cows were tested for their susceptibility to the development of oxidized flavor by the addition of copper to the milk. Group IV was the only one of the four groups of cows used in this experiment which produced milk susceptible to the development of this flavor to such an extent that it would be considered serious. Practically every cow in the entire herd, at one time or another during the time this investigation was conducted, produced milk that developed at least traces of oxidized

flavor, but each individual cow in Group IV consistently produced milk that was very susceptible to the development of this flavor. There was some variation in the degree of concentration of the flavor developed in the milk from the individual cows in the group, but every cow in this group consistently produced milk which was definitely susceptible to the development of the oxidized flavor.

**M17. The Relationship of Quality of Hay to the Development of Oxidized Flavor in Milk.** W. CARSON BROWN, A. H. VANLANDIGHAM AND CHAS. E. WEAKLEY, JR., West Virginia Agricultural Experiment Station.

Both carotene and ascorbic acid supplements in the feed have been shown to render milk non-susceptible to oxidized flavor. Since the carotene content of hay is a widely variable factor, it seemed advisable to determine the relationship between hay quality and oxidized flavor.

Eight Jersey cows were selected whose milk developed metal-induced oxidized flavor on the normal herd ration. Throughout the entire experiment carotene, ascorbic acid, and flavor determinations were made each week. The flavor determinations were made by adding none, 0.5, 1.0, and 1.5 p.p.m. of copper to pasteurized milk prior to storage for 3 days at 35 to 40° F. At the end of the storage period the samples were scored by 3 persons familiar with the flavor. After 5 weeks on a preliminary herd ration, all the animals were changed to a low-carotene ration. This ration consisted of 8 pounds brown leafy alfalfa hay (0.58 mg. of carotene per 100 grams) and 12 pounds of beet pulp per day as roughage, with a grain mixture of 100 pounds ground oats, 100 pounds wheat bran, 15 pounds cotton-seed meal, 3 pounds salt, and 2 pounds steamed bone meal fed according to production. After 4 weeks on the low-carotene ration the cows were divided into 2 groups producing milk about the same intensity of flavor. Group I was given the same ration as before except that the alfalfa was increased to 12 pounds per day while the cows in Group II were changed so that they received 12 pounds of bright, green alfalfa (4.30 mg. of carotene per 100 grams). After 5 weeks on this ration the cows in Group II had their ration supplemented by 2 pounds of alfalfa leaf meal (0.49 mg. of carotene per 100 grams) per day. Special care was taken to select hay of equal leafiness in both types of hay.

At the start and during the period of carotene depletion there did not appear to be any direct relationship between the carotene content of the milk and the intensity of the oxidized flavor developed. Even when the carotene content of the milk was reduced to about one-third of the quantity present at the start of the experiment, the oxidized flavor was not increased in intensity. However, the feeding of bright green alfalfa hay and alfalfa leaf meal resulted in an increased carotene content in the milk and a de-

crease in the intensity of the oxidized flavor developed. The level of the carotene in the milk after feeding the bright alfalfa was about the same as at the beginning of the experiment at which time the milk was susceptible to oxidized flavor. Even with the carotene content of the milk reduced to an extremely low level, no spontaneous development of oxidized flavor occurred. In general it appears that as the carotene content of the milk decreases the ascorbic acid increases. This relationship was rather general and daily fluctuations tended to obscure it.

From these results it would appear that there is no relationship between metal-induced oxidized flavor and the carotene content of the milk. Earlier work has shown that there is a relationship between carotene and ascorbic acid in the feed and oxidized flavor. Therefore it appears that susceptibility of milk to oxidized flavor is the result of a metabolic process involving carotene and ascorbic acid prior to the secretion of the milk, or the susceptibility is related to other substances accompanying these products in the feed.

**M18. The Effect of Feeding Cod-Liver Oil on the Goaty and Oxidized Flavors, and Vitamin C in Milk.** E. S. GUTHRIE, Cornell University.

This is the second report on the study of the effect of feeding cod-liver oil on the goaty and oxidized flavors, and vitamin C in milk. Last year four cows were fed cod liver oil for 59 days. This second report covers the feeding of cod-liver oil to six cows over a period of 164 days.

The cod-liver oil was administered in the first series of experiments by drenching. In the second set, the one this year, it was mixed in the feed of cows 3, 4, 5, and 6 during all of the oil feeding period. In case of cows 1 and 2 the oil was given both by feeding and drenching.

When it was found that the vitamin C would not climb to a high peak if the cod-liver oil was mixed with the feed, two of the least valuable cows were drenched. Cow 1 in this experiment was also No. 1 in the study of last year. She was the cow that responded most in the production of vitamin C in that first study.

The goaty flavor developed in the milk of only one animal in the group this year, whereas last year it was apparent in the milk of three of the four cows. This year, the goaty flavor appeared during the latter part of a feeding period and the first few days of the following rest period. This was also true in the three examples of last year. The vitamin C on the other hand was below average when the milk was goaty this year, whereas last year this flavor was noticeable when the vitamin C was present in abundance. It seems that there is no correlation between the presence of the goaty flavor and the amount of vitamin C.

There is an indication that cod-liver oil in the ration is a cause of the oxidized flavors.

When cod-liver oil was given at the rates of 0.5 ml. per kilogram weight of the cow the vitamin C remained constant. In case the cod-liver oil was administered by drenching at the same rate an increase of vitamin C was perceptible. Larger doses of cod-liver oil made distinct increases in Cow 1, reaching a high peak of 67 milligrams per liter of Vitamin C in the milk, when the average under normal conditions was about 27 milligrams per liter.

**M19. Resistance of Thermoduric Bacteria to Chlorine Disinfection.** A. C. MAACK AND M. J. PRUCHA, University of Illinois.

Occasionally difficulty is encountered in meeting the requirements for bacterial count in pasteurized milk because of the presence of thermoduric bacteria. The question has been raised as to the resistance of these organisms to chlorine disinfectants. The present study is an attempt to find the answer to that question.

The heat resistant organisms were found to be resistant to chlorine disinfection also. For example; one organism, non-spore forming, that required a temperature of 143° F. for 3½ hours to kill all of the bacterial cells also required chlorine strengths of 100 p.p.m. for 2 minutes or 20 p.p.m. for 5 minutes for its destruction.

It has been demonstrated that one of the main sources of these organisms are the utensils. Their resistance to chlorine disinfectants as well as to heat, may partly explain why they are present in milk in such large numbers as to be a problem in the dairy industry.

**M20. Is the Standard Plate Count a Proper Yardstick of Quality?** M. E. PARKER, Beatrice Creamery Company.

Today the standard plate count and possibly the number of coliform bacteria appear to occupy the center of the stage in the certification of sanitary quality of milk and its products. While we have come to accept certain numerical values as indicative of a proper sanitary quality, there is good reason to wonder if too much emphasis is not being placed on the quantitative significance of bacterial counts—particularly with respect to the pasteurized products. No one will deny that a "ceiling" for the total bacterial count in raw milk has practical value. The establishment of a "floor" for low counts in the raw or pasteurized milks, however, does not appear to be feasible. A good example of the false security possible in low bacterial counts is the widespread experiences with cappy or oxidized flavors in many a Grade A milk supply (both raw and pasteurized) during recent years.

Qualitative methods should prove valuable as they would tend to remove any elements of doubt regarding the true significance of practices which might otherwise be controversial. Mere bacterial numbers as enumerated

by the standard plate count are objectionable because of the variety of standards prevailing today which probably will be confusing no end with the projected changes in incubating temperatures and culture medium, not to mention the inherent inaccuracies of any cultural method of bacterial enumeration. Therefore, our plea is to pause and reflect. Consider quality control procedures objectively. Perhaps a good way to measure such intangibles as are involved in the quality control of dairy products—and we mean "quality control" in its broadest sense—is to apply qualitative methods in order to evaluate properly "quality." After all, John Ruskin was right when he said: "Quality is never an accident. It is always the result of intelligent effort."

**M21. Control of Sediment in Homogenized Milk.** A. J. HAHN AND P. H. TRACY, University of Illinois.

Sediment sometimes appears in the bottom of the bottle of homogenized milk twenty-four to forty-eight hours after bottling. This sediment generally contains leucocytes, epithelial cells, cell debris, protein material and dirt.

There is considerable variation in the cell content of milk from individual herds. Variations from a minimum of 203,840 cells per ml. to a maximum of 3,296,475 cells per ml. were observed, while the average count was 991,608 cells per ml. Samples from the same herds were again tested a week later with the result that the cell counts varied from a minimum of 121,030 cells per ml. to a maximum of 2,395,120 cells per ml. with an average of 890,295 cells per ml.

The extent of sedimentation in milk is related to the creaming ability of milk. When the creaming ability of milk was impaired or destroyed, either by homogenization or excessive heat treatment, the degree of sedimentation in that milk increased.

The degree of sedimentation in homogenized milk was increased by destabilizing the protein using calcium salts.

A chemical analysis, on a dry matter basis, of the sediment in homogenized milk showed that as the homogenization pressure increased there was also an increase in the percentage of protein contained in the sediment. The percentage of ash decreased with an increase in homogenizing pressure, while the percentage of ether soluble material underwent no changes.

From the standpoint of removing cells from milk, single clarification either using a clarifier unit or a separator unit and remixing the milk reduced the cell content on an average of 61.7 per cent and 51 per cent respectively. Filtration did not remove cells from milk appreciably. There did not seem to be any definite amount of cells removed from milk by clarification at any one time as data indicated variations throughout the entire clarifying period. The efficiency of cell removal by clarification increases with an increase in temperature.

Clarifying two or more times increases the extent of cell removal over a single clarification. Clarification after homogenization was more efficient than clarification before homogenization.

Pumping milk at slow speeds through an airtight clarifier results in greater clarification efficiency than pumping at the higher speeds.

Increasing the storage temperature of homogenized milk from 40° to 60° F. increased the degree of sedimentation of that milk. Placing the samples on a delivery truck for four hours where they could receive mild agitation did not increase the degree of sedimentation after 48 hours although the rate of sedimentation was increased to some degree.

**M22. A Study of the Effect of Added Iodine and Hydrogen Peroxide to Milk on the Enzymes.** MYER GLICKSTEIN, W. S. MUELLER AND J. H. FRANDSEN, Massachusetts State College.

A study of the effect of added iodine (both organic and inorganic) and hydrogen peroxide to milk was made in an effort to determine the possibilities of stimulating or inhibiting actions.

It was found that iodine and hydrogen peroxide in concentrations of as high as 100 p.p.m. in milk affects differently the activities of the enzymes studied.

Organic iodine stimulates catalase and peroxidase activities to a marked extent; inorganic iodine and hydrogen peroxide to a lesser degree. Lipase is adversely affected by the reagents used, with inorganic iodine showing the most drastic action.

Both types of iodine and hydrogen peroxide have a definite stimulating effect on gastric rennin, the action being most marked with organic iodine. Excessive use of these reagents produced inhibiting effects on the enzyme. Inorganic iodine has a paralyzing action on steapsin, whereas organic iodine and hydrogen peroxide have no significant effect on this lipolytic enzyme.

Inorganic iodine has a definite retarding action on the proteolytic enzymes, pepsin and trypsin. The action of organic iodine is less marked and that of hydrogen peroxide is negligible.

In general, it can be said that where there was a definite stimulation of enzymatic activity, organic iodine and, to a lesser degree, hydrogen peroxide were mainly responsible. In instances where inhibition took place, inorganic iodine was chiefly responsible and organic iodine and hydrogen peroxide had a materially reduced action, if any at all.

**M23. A Study of the Time-Temperature Relationships in the Pasteurization of Milk as Regards Creaming, Phosphatase and Bacterial Destruction.** R. F. HOLLAND AND A. C. DAHLBERG, New York Agricultural Experiment Station.

New and more rapid methods of heating, and the increasing use of high temperature-short hold pasteurization have created a need for a careful

study of the time and temperature relationships in the pasteurization of milk.

This investigation covers the temperature range 140° to 175° F. employing heating periods of only 2 to 10 seconds. The maximum holding periods which may be used without destruction of the creaming ability of the milk have been determined at each 5° interval over this range; also the minimum periods for the destruction of phosphatase and *Escherichia coli*.

The milk was brought to pasteurizing temperature in a thin walled tinned copper container 12 × 20 × 1 cms. in size held in a hot water bath. After the proper temperature was attained this container was transferred to a constant temperature bath for holding.

Cream layer volume was determined by placing 100 ml. of the milk in graduated cylinders in ice water and observing the depth of the layer 4 hours and 24 hours after pasteurization. Phosphatase tests were run by the Gilcreas and Davis modification of the Kay and Graham method and by the Neave modification of the Gilcreas-Davis test.

The milk was inoculated with a resistant strain of *Escherichia coli* before pasteurization and the surviving organisms determined by plating on violet red bile agar. Five formate-ricinoleate gas tubes were inoculated with 1 ml. of milk from each sample as a check on the plates.

When the results of these determinations were plotted on semi-logarithmic paper with time on the vertical axis on the logarithmic scale and temperature on the horizontal axis on the arithmetic scale, the points were found to fall on a straight line in each case.

The maximum times at which milk may be held without reduction of creaming ability varied from 80 minutes at 140° F. to 2.5 seconds at 170° F.

Bacterial destruction followed the creaming line exactly between 140° F. and 155° F. and then dropped slightly below at the higher temperatures.

The line for phosphatase destruction denoting proper pasteurization fell below the creaming line and ran parallel to it until a temperature of 165° F. was attained when it dropped off sharply.

**M24. The Relationship of Changes in the Chemical Composition of Milk to the Development of Mastitis.** A. H. VANLANDINGHAM, CHAS. E. WEAKLEY, JR. AND E. N. MOORE, West Virginia Agricultural Experiment Station.

Since mastitis does not develop simultaneously in all quarters of the udder, and since negative quarters in affected udders secrete what seems to be milk of normal composition, a study has been made of the normal variation in the chemical composition of milk from individual quarters of the same udder. It appeared that a study of this kind might be of considerable value in diagnosing chronic mastitis in individual quarters of the same udder.

Approximately 250 udder examinations on 40 pure-bred Holstein cows have been made. The percentage of chloride, lactose, total nitrogen, and non-casein nitrogen was determined on samples of foremilk from individual quarters. The chloride-lactose number and the casein number were calculated.

Physical examination and the following diagnostic tests on samples of foremilk were made in conjunction with the chemical studies: strip cup, brom thymol blue, Hotis test, chloride (colorimetric), microscopic examination of incubated milk, leucocyte count, and blood agar plate.

The foremilk from individual quarters free from mastitis was found to contain an average of 0.124 per cent chloride, and 4.79 per cent lactose. The average chloride-lactose number was 2.61 and the casein number 77.6.

The mean difference between quarters in normal udders free from mastitis was for chloride content 0.007 per cent, lactose 0.115 per cent, chloride-lactose number 0.184, and casein number 1.00.

In order to detect incipient stages of chronic mastitis by changes in the chemical composition of the milk from individual quarters, the quarter with the lowest per cent chloride and chloride lactose number or the highest per cent lactose or casein number is considered normal. For a significant difference between normal quarters and affected quarters there must be a difference of at least 0.02 per cent chloride, 0.36 per cent lactose, 0.60 for chloride lactose number, and 2.90 for casein number. This difference, for significance, is equal to the mean difference between normal quarters, plus two times the standard deviation of normal quarter differences.

Diagnosis of mastitis based upon quarter differences tends to eliminate difficulty due to changes in the chemical composition of milk from time to time as well as changes associated with advanced stages of lactation.

Individual quarters invariably showed bacteriological changes in the foremilk before a change in the chemical composition of the milk was apparent.

#### M25. The Determination of Copper in Butter. W. F. EPPLER AND B. E. HORRALL, Purdue University.

A review of the literature on the determination of copper in dairy products revealed that no one method was entirely satisfactory. This study has proven that for the determination of minute quantities of copper in dairy products, the colorimetric method using the neutral wedge photometer, was most satisfactory. The method as finally adopted is a combination and modification of the Williams<sup>1</sup> wet ashing method and the colorimetric method of Clifford and Wichmann.<sup>2</sup>

*Method.* To 50 gms. of butter in a 250 ml. beaker, 15 ml. C.P. nitric acid was added and slowly digested on a steam bath until both layers were clear.

<sup>1</sup> J. Dairy Research, 3, 1931.

<sup>2</sup> J. A. O. A. C., 19, No. 1, 1936; 22, No. 2, 1939.

The covered beaker was then cooled in a refrigerator until the fat layer congealed. The congealed fat layer was punctured and the nitric acid layer drained into a 500 ml. Kjeldahl flask. The fat was washed by adding 50 ml. of glass distilled water, heated until the fat melted with occasional swirling of beaker, then cooled and the water layer added to the nitric acid in the Kjeldahl flask. This procedure was repeated three times. Ten ml. of concentrated sulfuric acid were added to this mixture and slowly heated on a Kjeldahl digestion apparatus until the contents assumed a black frothy consistency, after which the flask was removed from the rack and allowed to cool. Then four to five drops (about 0.2 ml.) perchloric acid (70 per cent) were added with caution. After the decomposition of organic matter, the heat was increased to volatilize the excess acid and this continued until about three ml. remained. After cooling, the residue was transferred to a 250 ml. separatory funnel with small portions of hot distilled water and neutralized with ammonium hydroxide using litmus paper (any great excess was avoided). Ten ml. of 15 per cent solution of citric acid were added and the whole diluted to 90 ml. When at room temperature, 10 ml. of 0.1 per cent sodium-diethyl-dithio-carbamate solution were added and the contents well shaken. To this mixture, 20 ml. of redistilled carbon-tetra-chloride were added and the mixture shaken vigorously until complete extraction of the color by the solvent was obtained.

The carbon-tetra-chloride was filtered into the standard tube and the color measured with the use of a neutral wedge photometer. The light filter used was a Wratten No. 62 Hg. Green ( $530 \text{ m} \mu$ ) mounted in B glass. The tube was 150 mm. in length and 12 mm. in diameter.

*Results.* The data show that a high percentage of recovery is possible when known concentrations of copper are carried through the entire method.

The method permits the use of a large sample without the possibility of reagent contamination.

The photometer used is sensitive through a color range of 5 to 50 p.p.m. of copper.

#### M26. The Uniformity of Butter Composition as Related to Type of Churn. S. L. TUCKEY AND P. H. TRACY, University of Illinois.

The production of butter of uniform composition is one of the essentials of good plant management. One of the important factors in the production of butter of uniform composition is the type and construction of the churn.

Even though churning load, churning temperature, butter granule size, wash water temperature, and other important factors may be properly controlled, it will be impossible for the operator to produce butter of uniform composition if the churn is of such construction that it permits excess water to remain at one end or the center of the churn.

For this analytical study butter samples were obtained from several types of churning under commercial operating conditions. For comparison,

four samples were secured from each end and from the center of each churn. In this experiment over 200 samples were analyzed by the Kohman method.

Our data serve to stress the importance that each operator know the characteristics of his particular churn since no particular type or make of churn was found to produce butter of uniform composition consistently. New workerless churning were found in which the butter taken from the two ends varied over 1 per cent in composition. Also workerless churning were found that produced butter of uniform composition; samples taken from the two ends showing a variation of only 0.1 per cent. These statements may be applied also to worker type churning. Some worker churning which had been remodeled to act as workerless churning produced butter of greater uniformity than that produced before the rollers were removed.

**M27. Changes in the Bacterial Flora of Butter.** C. A. WILSON AND M. J. PRUCHA, University of Illinois.

Bacterial deterioration of butter is still largely an unsolved problem of the dairy industry, although it has been the subject of a considerable amount of research.

The following study, consisting of observations of the changes in the bacterial flora was made in an effort to throw additional light on the subject.

Butter samples were made from sour, neutralized cream which had been processed by three different methods of pasteurization. Studies were made on the raw cream, pasteurized cream, fresh butter, and butter stored for 3 weeks at 65° F. and for 4, 8, and 12 weeks at 40° F.

The method of study consisted of making agar plates of suitable dilutions. Usually the plates that had about 50 colonies were selected and all the colonies were picked and inoculated into sterile litmus milk. The microbial flora was divided according to the reaction in the litmus milk after two weeks of incubation at 90° F. into the following groups:

1. Fast acid formers—milk clabbered.
2. Slow acid formers—milk not clabbered.
3. Alkali formers.
4. Sweet curd formers.
5. Peptonizers.
6. Inert—no visible reaction.

The microbial flora in the raw cream consisted chiefly of the rapid acid producing coagulating organisms. After pasteurization, the rapid acid organisms decreased but the flora was still predominantly acid forming. The slow acid formers became more numerous on percentage basis. The percentage of acid formers in the raw and pasteurized cream tended to remain quite constant.

In the freshly made butter, the percentage of the acid formers decreased and the percentage of the alkali-formers, of the peptonizers and of the inert increased.

Three weeks storage of the butter at 65° F. resulted in an almost complete elimination of the acid forming organisms. The microbial flora consisted mostly of the alkali-formers and of the inert types.

The flora of the butter stored at 40° F. tended to remain of the same kind as that of the fresh butter.

No marked differences were observed between the bacterial flora of the cream pasteurized by the three different methods or between the butter made from these creams.

**M28. Some Preliminary Observations on the Effectiveness of Propionates as Mold Inhibitors on Dairy Products.** J. D. INGLE, Swift & Company Chemical Laboratories.

Several series of tests were run on fresh cut blocks of natural cheese wrapped in tinfoil, moisture-proof cellophane, or pliofilm. The samples were either dipped in propionate solutions or the wrappers sponged with the solutions. The results indicated that sponging the wrappers has little effect in holding down surface mold. Samples dipped in 8 per cent propionic acid held up about twice as long before showing visible mold as compared to the controls. Eight per cent calcium and 8 per cent sodium propionate treated samples were somewhat better than the controls but did not approach the 8 per cent propionic acid in effectiveness.

Tests made indicate that small percentages of sodium or calcium propionate incorporated into processed cream cheese are only slightly effective in holding down surface mold. The most effective method of holding down surface mold on cold packed cream cheese was found to be that of waxing the wrappers with a wax containing propionic acid.

Tests made on unsalted butter stored at 60° F. and 100 per cent humidity indicated that wrappers containing either 6 per cent calcium propionate or 12 per cent sodium propionate greatly inhibited the growth of mold on the surface of the butter. Wetting the propionate impregnated wrappers before wrapping gave better results than using the dried impregnated wrappers.

There seemed to be little difference in effect between the parchments treated with sodium and calcium propionates.

**M29. Propionic Acid and Its Calcium and Sodium Salts as Inhibitors of Mold Growth.** J. C. OLSON AND H. MACY, University of Minnesota.

A study has been made to determine the effectiveness of propionic acid, calcium propionate and sodium propionate in inhibiting the growth of various species of mold on the surface of butter and in culture media.

It has been found that it requires less calcium propionate by weight, than sodium propionate to inhibit the development of molds on media and on the

surface of butter wrapped with parchment treated with solutions of the propionates. Further, it requires a much lower concentration by weight of propionic acid to bring about the same degree of inhibition.

The final pH of media containing either of the salts is an extremely important factor in restraining mold growth, for example, with a two per cent concentration of sodium propionate in potato dextrose agar, the reaction was approximately at pH 7.00 and relatively rapid and abundant mold growth occurred. When the medium was brought to pH 6.1 by the addition of lactic acid no growth occurred in five days.

Some difference in the tolerance of several genera of molds to propionic acid and its sodium and calcium salts has been noted. Of those studied, *Penicillium* species showed the greatest tolerance.

In an attempt to determine the agent actively responsible for the inhibitory effect of the salts and the acid, several observations have been made. In potato dextrose agar to which had been added sufficient propionic acid to give a 0.009 M concentration the growth of *Hormodendrum cladosporoides* was relatively abundant; when 0.4 gm. of sodium propionate was added to 100 ml. of such acidulated medium, growth was completely checked, but when 0.4 gm. of sodium propionate alone was added to 100 ml. of potato dextrose agar containing no propionic acid there was abundant growth. This observation and other experimental results point to the possibility that the undissociated propionic acid is responsible for the growth inhibition.

### M30. Some of the Factors Affecting the Phosphatase Values of Butter.

W. H. BROWN, Purdue University.

The application of the phosphatase test to sour cream butter presents a more complex problem in the interpretation of the results than the application of the test to sweet products. Studies were made to determine some of the reasons for inaccuracies that may occur which are not directly due to the technique of the test. The methods used were those developed by Scharer.

The concentration of the phosphatase enzyme in cream has been recognized. There is a further concentration of the enzyme in butter during the buttermaking process. This fact may give rise to a positive phosphatase value for butter, if the suggested milk standards are used, even though the cream gave a negative test.

An increase of the phosphatase value of butter from a negative to a positive test during a storage period of 10 days at 60° F. has been encountered in approximately 10 per cent of the samples analyzed. This increase in phosphatase value also occurred at temperatures considerably lower than 60° F. However, the values obtained on butter samples held at 0° F. remained constant for three years. The possibility that this increase might be due to the production of the phosphatase enzyme by micro-organisms has

been investigated. Many micro-organisms were isolated which are capable of producing the enzyme in milk and cream, but none of these organisms produced the enzyme in butter. By increasing the incubation period in determining the phosphatase value of fresh samples, it was possible to predict, in many cases, those samples which changed from negative to positive during storage.

Slow cooling of the cream after marginal pasteurization has been found to affect the phosphatase reaction of the cream and butter.

It was found that the enzyme phosphatase is not acid tolerant. As the development of acidity in raw cream increases, the destruction of the phosphatase enzyme increases.

**M31. Effect of Salt on the Keeping Quality of Cream.** W. J. CAULFIELD,  
F. E. NELSON AND W. H. MARTIN, Kansas Agricultural Experiment  
Station.

In each of a series of four trials, four samples of 30 per cent cream to which salt in quantities equal to 0, 7, 10, 13 and 16 per cent of the weight of the fat-free serum was added, were held at 60, 70, 82, and 90° F. for 10-day periods. Changes in acidity, formol titration and grade were followed, observations being made at 1, 2, 3, 4, 5, 6, 8 and 10 day periods. Changes in the bacterial flora of two samples were followed by direct microscopic observations. All control samples deteriorated rapidly, and at 80 and 90° F. became unlawful within five days. At 60° F., 7 per cent salt kept the cream from going below first grade in all samples, but at higher temperatures this concentration was not sufficient to keep the cream from becoming second grade or lower. Salt in 10 per cent concentration kept the cream from changing from sweet to first grade at 60 and 70° F. but was considerably less effective at 82 and 90° F. where much second grade cream resulted when this salt concentration was used. Salt in 13 and 16 per cent concentrations kept the cream sweet through a five day period at all temperatures, with the exception of two samples, and through the 10 day period at 60 and 70° F. with but one exception. At 82° F. these two salt concentrations kept all samples from becoming second grade, but at 90° F. second grade cream was obtained in about half of the samples. The data from acidity and formol titrations and the bacteriological observations corroborate the results of the organoleptic grading.

In each of a series of four other trials, the addition of 13 per cent salt on a serum basis to the cream after holding at 70° F. for 3, 4, 5, and 6 day intervals did not prevent further deterioration during the remainder of a 10 day storage period.

In three additional trials, creams to which 13 per cent salt on a serum basis was added and control lots of the same creams without salt were held at 70° F. for 10 days. The resulting creams were then neutralized if neces-

sary, pasteurized at 150° F. for 30 minutes and churned, unsalted butter being made. After storage the control creams were all low second grade, while the creams to which salt had been added were all on the border line between sweet and first grade. Acidity and formol titrations and bacteriological results again corroborated the organoleptic findings. The fresh butter from the creams to which salt had been added graded 92, 92, and 92.5, while the butters from the control creams graded 87, 90, and 90 respectively. No change in score occurred after storage at -10° F. for 60 days, indicating no greater tendency for chemical change in the butters from the salted creams.

Unsatisfactory butterfat tests were obtained on the cream to which salt had been added when the usual Babcock procedure was employed. When a modified procedure was used, results which agreed favorably with the calculated butterfat percentages were obtained.

**Summary.** The results indicate that the deterioration of cream, held without the benefit of adequate cooling, may be retarded by the addition of salt. The amount of salt necessary will depend upon the time and temperature of storage. The addition of salt will not prevent further change in cream which has already undergone appreciable deterioration and thus the method is limited largely to farm use.

### M32. The Chemical and Bacteriological Changes in Brick Cheese During Manufacture. J. C. GAREY, E. M. FOSTER AND W. C. FRAZIER, University of Wisconsin.

Changes in the numbers of bacteria in brick cheese were followed by means of cultural and direct microscopic methods.

When *Streptococcus lactis* was the only starter used, no growth of this organism took place in the vat when cooking temperatures were 104° F. or 112° F. At the lower cooking temperature, the numbers of *S. lactis* increased slowly until the fourth to sixth hour after dipping, then multiplication became very rapid. The maximum numbers of bacteria were attained 20 to 24 hours after dipping. At the higher cooking temperature, the beginning of rapid growth was delayed until the sixth to eighth hour after dipping. Maximum numbers again were reached 20 to 24 hours after dipping. At that time the pH of the cheese varied from 4.9 to 5.1.

When only *Streptococcus thermophilus* was used as starter, growth of this organism began almost immediately, continued rapidly during curd-making (cooked at 104° F.) and in the dipped curd until the third or fourth hour after dipping. Maximum numbers were reached 10 to 12 hours after dipping. At that time the pH varied from 5.2 to 5.35. When the cooking temperature was raised from 104° F. to 112° F. the period of rapid growth was longer and maximum numbers were higher.

When both *S. lactis* and *S. thermophilus* were used (cooking temperature 104° F.) and their proportions varied, a 1:1 ratio (0.5 per cent each) was

found to produce the most desirable type of brick cheese. When this proportion was used, the cessation of growth of *S. thermophilus* and the beginning of rapid growth of *S. lactis* overlapped. This resulted in a steady growth of starter bacteria throughout the making process until the maximum numbers were reached 20 to 24 hours after dipping. At that time the pH was 5.0 to 5.15.

The moisture at two weeks in all of the above cheese ranged from 37.5 to 40.0 per cent.

By means of a washed-curd method, sweet cheese was made with 42 to 44 per cent moisture at two weeks. The washing process removed about 40 per cent of the lactose.

In a study of the development and prevention of "early gas" it was found that 2.5 per cent of starter (0.5 per cent *S. thermophilus* and 2.0 per cent *S. lactis*) would prevent "blowing" when conventional manufacturing methods were used. This was not true with the washed curd methods, where it was found that 3.5 per cent starter (0.5 per cent *S. thermophilus* and 3 per cent *S. lactis*) failed to prevent "blowing." This defect could be prevented in the washed curd process by cooking to 120° F. and using 0.3 per cent *S. thermophilus* and 0.5 per cent *Lactobacillus bulgaricus*. Gassiness in the cheese was also prevented by pasteurization of the milk.

The bacteriological study of the interior of brick cheese during ripening revealed that the *S. lactis* types of bacteria eventually predominated. The *S. thermophilus* types decreased rapidly in numbers even when *S. thermophilus* was added. A few species of lactobacilli, notably *Lactobacillus casei*, appeared in the raw milk cheese after about two to three weeks and gradually increased in numbers thereafter.

**M33. The Control of Abnormal Bacterial Fermentations in the Manufacture of Swiss Cheese.** LLOYD A. BURKEY, MORRISON ROGOSA AND ROBERT R. FARRAR, Bureau of Dairy Industry, U. S. Department of Agriculture.

A study of bitter flavor and reddish spots in Swiss cheese indicates that these abnormalities are caused by equipment contamination.

Bitter, peppery, and other off-flavors in Swiss cheese were associated with the presence of large numbers of aerobic spore-bearing bacteria. These bacteria were isolated from a sample of bitter cheese which was typical of over one third of the cheese made in one section of the country. The isolated cultures are similar in many respects to *Bacillus vulgatus* and *Bacillus mesentericus*. They are actively caseolytic and produce a distinct bitterness in sterile whole milk but only a slight bitterness in sterile skimmed milk.

Studies made of these cultures under various conditions, including experimental work in laboratory Swiss cheese, show that they are resistant to high temperatures, will grow and attain numbers well over a million per

gram in cured cheese, persist several months in the cheese making equipment, and can be eliminated from this equipment only by severe sterilization methods.

Bitterness in Swiss cheese from this cause is believed to be associated with high moisture. Factors which inhibited the development of the bitter forming bacteria in Swiss cheese were:

- (1) The use of an active culture of *Streptococcus thermophilus* with a correspondingly rapid production of acid at the period three hours after dipping.
- (2) Adherence to a making process of high cooking temperature (over 53° C.), short foreworking, and a long "stirring out" period.
- (3) Addition of salt to the kettle milk and heavy salting during the curing process.

The presence of reddish or brown spots in cured Swiss cheese was found to be caused by the development in the cheese of a contaminating type of propionic acid bacteria persisting in cheese making equipment. In several instances, crevices, roughness, or bad joints in equipment apparently provided places of lodgment for these contaminants.

Suggestions for the prevention of losses caused by these defects are as follows:

- (1) Elimination of roughness or places of lodgment for bacteria in all equipment.
- (2) Daily thorough cleaning of all equipment.
- (3) Occasional sterilization of all equipment by means of prolonged hot water treatment or use of an efficient chemical disinfectant.
- (4) The use of pure culture starters of proper activity.

It is believed that laxity in care of cheese making equipment and failure to use active starters of known purity, are responsible for other defects in Swiss cheese associated with abnormal eye formation.

#### M34. The Effect of Heat-Treatment of Milk on the Activity of Swiss Cheese Starters. M. E. TYLER AND H. H. WEISER, The Ohio State University.

The normal ripening process involved in the manufacture of Swiss cheese depends largely on the biochemical activity of the micro-organisms used in the starter. Under factory conditions, the heat-treatment of the milk used in the preparation of the bulk starter may vary widely and may influence the activity of the starter, assuming that other environmental factors are properly controlled. It was decided, therefore, to study the effect of various heat-treatments of the milk, made immediately prior to inoculation, on the activity of the starter organisms. In this study, 18 strains of streptococci and lactobacilli used in the manufacture of Swiss cheese were employed.

Fresh, raw, whole milk was dispensed in 75 cc. amounts in large, sterile

test tubes, and each tube of milk was heated at a given temperature for a given time interval as follows:

Series I. Samples of milk heated at 80° C. for 1, 2, 3 and 4 hour periods.

Series II. Samples of milk heated at 100° C. for 1, 2, 4 and 5 hour periods.

Series III. Samples of milk heated at 120° C. for 15, 30, 45 and 60 minute periods.

At the end of the heating period the milk was cooled to room temperature and the redox potential was determined, the hydrogen-ion concentration remaining fairly constant. The saturated calomel half-cell, a Model 3C Coleman potentiometer and bright platinum foil electrodes were employed in the determination of the potential. The samples were then inoculated with a Swiss cheese starter culture, amounting to 1 per cent of the volume of each sample. Incubation of these samples was at 37° C. for 24 hours.

The activity of the various starter organisms was determined by titrating the cultures for acidity, using 10 cc. amounts, 0.10 N NaOH, and phenolphthalein as the indicator. The titration was carried out after the starters were incubated at 37° C. for 12 and 24 hours.

It was noted that as the heating period was prolonged at 80° C., 100° C., or 120° C., the Eh curve showed a significant decrease. Milk heated at 80° C. showed very little change in the acid production. When the milk was heated at 100° C. for 2 or 4 hours the maximum amount of acidity was produced by all the cultures studied; a minimum amount of acid was formed in milk heated for 1 hour, while the 5-hour heating period showed considerable variation in the amounts of acid produced by the various organisms. The greatest amount of acid that was formed in the milk heated at 120° C. occurred in the 30 or 45 minute periods, the 15 and 60 minute periods exhibiting a wide range in acid production.

It appears that the use of proper time and temperature of heating the milk employed in the preparation of bulk starters has a favorable influence on the activity of the various starter organisms used in the ripening of Swiss cheese. This may be due to the fact that the Eh of the milk is lowered, thus providing a more satisfactory cultural environment for the micro-organisms concerned.

**M35. The Standardization of Fat in Swiss Cheese and the Relationship of Fat to Quality.** GEORGE P. SANDERS, ROBERT R. FARRAR, FRED FEUTZ AND ROBERT E. HARDELL,\* Bureau of Dairy Industry, U. S. Department of Agriculture.†

1. *Estimation of percentage of fat in dry matter.* For securing results on composition promptly without plugging the cheese, a method has been

\* Resigned March, 1940.

† In cooperation with the Departments of Dairy Industry of the University of Wisconsin and the Ohio State University.

devised for preparing pressed samples of kettle curd for analyses. Samples of curd are taken from the kettle, just prior to dipping, by means of strainer-bottom dippers. Two such samples are placed in a perforated-bottom, metal cylinder, a 1000-gram weight is inserted, and excess whey is thus squeezed out. The composition of the sample approximates that of the cheese. Its firm, compact condition, like that of the cheese, permits it to be cut into strips for the fat test. In 355 sets of samples, values of fat in dry matter of pressed curd were within 1 per cent of those found in the corresponding cured cheese in 70 per cent of the cases, and within 2 per cent in 95 per cent of the cases. For efficient standardization it is recommended that, in addition to frequently analyses of pressed curd and cured cheese, fat tests be made of milk samples from every kettle.

*2. Relationship of percentage of fat in dry matter to quality.* Tabulations of analytical and grade data for 632 cheeses, sampled in 39 factories principally in Wisconsin and Ohio, indicate that highest average quality was found in cheese containing 45-46 per cent fat in dry matter, and that cheese in which the values exceeded this range was somewhat superior to that which contained less than 45 per cent fat in dry matter. In controlled comparisons on 30 pairs of laboratory cheese made from high-solids milk, yielding cheese that was rather firm in texture, cheese containing an average of 48 per cent fat in dry matter was superior to that containing less than 45 per cent.

### M36. Improving the Quality of Swiss Cheese Through Applied Research and Technical Control. ROBERT R. FARRAR, Bureau of Dairy Industry, U. S. Department of Agriculture.

Improvements were made in the quality of the Swiss cheese produced by a large factory in Idaho during the four summer months of 1939, using the methods recommended by the Bureau of Dairy Industry.

The proportion of low quality or grinder cheese was reduced to 28.6 per cent for the period as compared with 63.3 per cent for the same four months the previous year. During August, the last month of this project, the grinder cheese amounted to 11.6 per cent of the total make as compared with 58.1 per cent the previous year. Under the conditions then prevailing the returns were 33 per cent more for a C grade cheese than for a grinder cheese.

Improvement in the quality of the cheese was effected through (1) the introduction of pure culture starters and improved methods of starter propagation, (2) improvement of the quality of the milk, (3) changes in the manufacturing methods used, including composition control, and (4) improvement in curing-room management.

The improvement in cheese grades effected during late May and during the first several weeks of June can be attributed primarily to starter improvement.

As a result of a milk quality improvement program among the producers the individual methylene blue reduction time was increased from an average of 1.5 hours on May 30 to 5.5 hours on August 10 when general conditions were not as favorable. The temperature of the milk at the intake averaged 7° F. lower on this latter date. An average methylene blue time of 5.5 hours was necessary to obtain a 3-hour reduction time in the kettle milk. Kettle milks having approximately a 3-hour reduction time yielded the highest average quality cheese.

In an attempt to produce the highest average quality cheese the milk was sorted at the intake on the basis of methylene blue time, acidity, and odor; the "poor" milk cheeses graded 78.6 per cent grinders for the 4 months and the "good" milk averaged 19.5 per cent grinders. For the month of August the "poor" milk cheese graded 70.6 per cent grinders as compared with 4.3 per cent for the "good" milk cheese.

The manufacturing methods were changed to meet the changing characteristics of the milk and to produce as nearly as possible a cheese containing less than 40 per cent moisture and at least 45 per cent fat in dry matter. Controlling the acid development at 3 hours after dipping within the range of pH 5.90 to 5.70 was effective in preventing "pressler" cheese.

Changes were made in curing-room management to slow up the rise of the cheese and to provide more salt. The relative humidity of the curing rooms was corrected.

Shrinkage during the usual two-month curing period, through changes in manufacturing methods and curing room humidity, was reduced to a normal figure of 7.59 per cent, as compared with a former extreme of 14 per cent.

Similar improvement in cheese quality was effected at a Pennsylvania Swiss cheese factory where visits of several days duration were made at six-week intervals.

#### **M37. Relation of Salt Content to Bitter Flavor Development in Cheddar Cheese. S. L. TUCKEY AND H. A. RUEHE, University of Illinois.**

The flavor of cheddar cheese is the result of the action of several factors, the more important of these being bacterial development, acidity development, moisture content, and salt content. For several years the authors have judged cheese at the Illinois State Fair, and one of the most common defects in these cheese samples was the presence of a bitter flavor. It was also noted that this bitter flavor was not necessarily associated with characteristic acid defects. Cheese made at the College Creamery also frequently developed this characteristic bitter flavor. In an attempt to determine the cause and, if possible, the remedy for this defect, a study was undertaken to determine the relationship between the salt content of cheese and bitter flavor development.

Ten lots of cheese were made using 3000 pounds of 3.5 per cent-3.7 per cent milk in each lot. The procedure was such that the whey was drawn at 0.14 per cent acidity, the curd milled at 0.5 per cent acidity, and salt was added at the rate of 2.6-2.7 pounds per 1000 pounds of milk. The finished cheese had a moisture content of 36-37 per cent. The salting period lasted 60 minutes; however, at regular intervals during this time part of the cheese was taken from the vat and packed in longhorn molds. These samples were used for analyses and judging. Salt determinations were made by the distillation method of Whitmore and Overman. Our results show that:

1. The average salt content of cheese salted for 20 minutes was 1.33 per cent; for 40 minutes was 1.60 per cent; and for 60 minutes was 1.70 per cent.
2. The cheese salted for 20 minutes and 40 minutes developed a lower pH than did the cheese salted for 60 minutes although not low enough to develop acid defects in the body.
3. The cheese salted for 20 minutes developed a bitter flavor, and this flavor was detected often in the cheese salted for 45 minutes, but the flavor was not present in the cheese salted for one hour providing the salt content was 1.7 per cent or more.
4. There is a close correlation between a low salt content of cheddar cheese and a characteristic bitter flavor.

**M38. More Accurate Determinations of Volatile Fatty Acid and Other Changes as a Means to Study Cheddar Cheese Curing.** J. C. MARQUARDT AND A. C. DAHLBERG, New York Agricultural Experiment Station.

An investigation has been started to develop analytical procedures to follow and interpret changes in the curing of cheddar cheese. It is eventually planned to use these methods to obtain a better understanding of the causative factors for differences in the curing of raw and pasteurized milk cheese, the seasonal variations in curing, and the role of lipase in cheese curing.

The investigation up to the present time has been devoted to the development of suitable procedures for following fat changes in the curing of cheddar cheese. Literature reviews have indicated that methods commonly used are subject to irregularities. It has been established that the amounts of volatile fatty acids obtained by extracting fat by pressure or water-ether solution from the cheese are far greater than those which are obtained directly from the cheese by steam distillation. The studies have also shown the difficulties encountered collecting certain quantities of distillate and expressing for comparative purposes the values obtained with these amounts.

It has been found that salt, moisture, protein, and fat determinations are essential on all cheese and the component parts used in the studies. These are used mainly to augment analyses which follow.

In the studies we have determined the amounts of cheese to use depending upon the age of the cheese for steam distillation so that all of the volatile fatty acids will be obtained in the first 2000 cc. of distillate. Similarly suitable amounts for the extracted fat for steam distillation have been determined. All of these values are finally reduced to comparable terms. It has been possible to perfect methods to show that the amount of volatile fatty acids which can be steam distilled from pressure extracted fats are two or more times greater than those obtained by steam distilling the cheese. Higher values are also obtained when water-ether extracts of the cheese are steam distilled.

The purpose of the study up to the present time has been to develop procedures whereby the fat changes in curing cheese can be more accurately studied.

**M39. Effect of Lipolytic Enzymes on the Ripening of Cheddar Cheese.**

C. B. LANE AND B. W. HAMMER, Iowa Agricultural Experiment Station.

Studies have been continued on the development of flavor in cheddar cheese. Since the relatively high acid numbers obtained on fat from ripened cheese suggest the importance of a limited fat hydrolysis, the effect of lipolytic enzymes on cheese ripening was studied.

Lots of cheese were made in which lipolytic enzymes were added to the pasteurized milk used for cheesemaking. Control cheese not containing the enzymes were made from the same original lots of milk. The cheese were examined organoleptically several times during a ripening period of 3 months.

Pancreatin had a rather undesirable effect on the cheese flavor even when added in very small concentrations. A disagreeable rancid condition was regularly produced and persisted during the entire ripening period.

Desiccated, bovine mammary tissue or water extracts of it appeared to have a desirable effect on the cheese ripening. Different lots of tissue varied considerably in lipolytic activity so that it was difficult to determine suitable amounts to employ. Satisfactory results were commonly obtained with 25 to 35 gm. of tissue or the equivalent in extract to 1000 pounds of milk. Cheese made with the tissue or its extract usually developed "cheddar" flavor more rapidly than the control cheese, and the body and texture were often considered more desirable. When a relatively large amount of tissue (100 gm. to 1000 pounds of milk) or when a smaller amount of highly lipolytic tissue was employed, a rancid flavor sometimes developed in the very young cheese, but it disappeared as the cheese aged.

Standardization of extracts of mammary tissue on the basis of lipolytic activity is being attempted.

**M40. The Purification of Rennin.** C. L. HANKINSON\* AND L. S. PALMER,  
University of Minnesota.

Two liters of Hansen's rennet extract were adjusted to a pH of 4.5 with 7.0 ml. of concentrated HCl. A precipitate formed readily and was centrifuged for 20 minutes at 2000 r.p.m. The supernatant liquid was poured off and the precipitate was dispersed in sufficient 16.7 per cent NaCl solution (20 gm. NaCl per 100 ml. H<sub>2</sub>O) to make one liter volume. The pH was adjusted to 6.0, whereupon the precipitate all dissolved. The pH was again adjusted to 4.5 and the suspension centrifuged for 20 minutes. The precipitate was dissolved in 16.7 per cent NaCl at pH 6.0 and made up to 500 ml. volume with the solvent. There was some sediment at this point. This was centrifuged out since previous work had shown it to be relatively inactive. The pH was then adjusted to 4.5 and the suspension centrifuged for 20 minutes. The precipitate was dissolved in the 16.7 per cent NaCl, made up to 250 ml. with solvent and the pH adjusted to 6.1. Again there was considerable sediment which was centrifuged out. Activity determinations were made on the final liquid and on the original rennet extract. The solution was stored in a cold room at 2° C. Portions were dialyzed as needed.

By following the activity per unit weight of dry material in the precipitate and supernatant liquid, it was found that the most active rennin preparation could be obtained by the above procedure. Precipitation at varying pH with varying salt concentrations and different kinds of salt led to this procedure. It was found that the most active rennin material behaved as a globulin, (1) being soluble in dilute salt solution, (2) insoluble in saturated salt solutions, (3) precipitating at the isoelectric point pH 4.6, and (4) precipitating upon electrodialysis. This is in contrast to reports in the literature that rennin is an acid albumin (Fenger, 1923), thioproteose (Tauber and Kleiner, 1932) or some non-protein material (Lüers and Bader, 1927). The reddish brown or coffee-brown color is not associated with the rennin activity as believed by Richardson and Palmer. Most of this colored material is left in the first supernatant liquor at pH 4.5.

This procedure has the further advantage of separating the peptic or proteolytic from the purely rennin active material. Most of the peptic activity is left in the first supernatant liquor at pH 4.5. Thus there may be some association between the pepsin and the reddish brown colored material.

This rennin preparation shows an increase in activity of nearly four times that of the original extract while 92.5 per cent of the proteolytic activity has been removed. It was found possible to store the enzyme in 16.7 per cent NaCl solution at 2° C. with very little loss of activity in three months.

**M41. The Effect of Standardizing the Acidity on the Methods and Physical and Chemical Properties of Cottage Cheese and Cul-**

\* Present Address: Carnation Research Laboratories, Milwaukee, Wisconsin.

**tured Buttermilk.\* L. E. MULL AND W. H. E. REID, Missouri Agricultural Experiment Station.**

In this investigation, a study was made of the effect of adjusting the acidity at different steps in the manufacturing process upon the physical and chemical properties of cottage cheese and cultured buttermilk.

It was found that cottage cheese curd washed in water containing variable increments of standardizing agent produced a clean, sweet, mild flavored curd. High concentrations of standardizer had a tendency to produce a weak bodied, slick curd, and in some instances a gelatinous material formed around the curd particle.

Adjusting the acidity in the cream used in creaming the curd enhanced the flavor of cottage cheese. The curd, creamed with low acidity cream, maintained a higher pH and a lower acidity throughout the storage period than did curd creamed with normal acidity cream.

Adjusting the acidity in the skim milk before setting, and varying the amount of starter and rennet improved the flavor and body, and reduced the time from setting to cutting of the curd to approximately two and one-half hours.

The use of an excessive amount of standardizer in the storage water resulted in an undesirable flavored curd with an inferior keeping quality.

A clean, full, mellow flavor, and a smooth body of a desirable viscosity was obtained in cultured buttermilk by adjusting the acidity in the milk before setting. There was no indication of wheyng-off at the end of a seven-day storage period. Adjusting the acidity in the milk, increasing the amount of starter, and raising the setting temperature produced a high quality cultured buttermilk in approximately five hours.

**M42. The Use of Homogenized Milk in the Manufacture of Cottage Cheese. D. W. GLOVER AND L. H. BURGWALD, Ohio State University.**

Since the advent of homogenization, dealers selling homogenized milk have been confronted with a problem of utilization of returns. One method for utilizing returns of bottled homogenized milk is to manufacture it into cottage cheese.

Experimental batches of cheese were made using homogenized milks of various butterfat content. The trials were made in groups ranging in number from three to five, each group including one batch from skimmilk for a control. It was found advisable to add calcium chloride to the homogenized milk to increase the coagulability; one cubic centimeter of a saturated solution was added per 100 pounds of milk in the vat. A setting temperature of 70° F. and the acid-rennet method of coagulation was used (1.0 cc. rennet per 1,000 pounds milk). Fat and solids determinations were made using the Mojonnier method.

\* Missouri Experiment Station, Journal Series No. 626.

Keeping quality of homogenized milk cheese and creamed curd of equal butterfat content were compared by storing the samples at 45° F.; samples were judged for flavor daily or every two days. In every trial, the same amount of cream was added per 100 pounds of curd, and the butterfat content of the cream was varied to meet the fat requirements. Homogenized milk cheese of a given butterfat content exhibited better keeping qualities at 45° F. than cheese from skimmilk brought up to an equivalent butterfat percentage by adding cream pasteurized at 143° F. for thirty minutes.

Results of preliminary trials in which four, three, and two per cent homogenized milk were used indicated that it is not advisable to use milk exceeding two per cent butterfat content. Cheese resulting from these higher fat content milks were very high in butterfat and were mushy in spite of the low moisture content.

The coagulum may lack the gel-like property exhibited by that of coagulum from skimmilk; consequently, in cutting the curd, it is necessary to exercise care in order that the curd not be broken up into uneven sized particles.

Fat losses in the whey were greater in cases where milk of higher fat content was used. Analyses of whey samples from trials in which milk having two per cent or less butterfat content did not show excessive fat loss in the whey (0.04 to 0.109 per cent).

Solids tests on the whey obtained from various trials show that there is little difference in solids in the whey when using milks of different butterfat content (6.50 to 6.92 per cent).

The average yield of curd increased as the butterfat content of the milk used increased. The butterfat content of the cheese increased as the per cent butterfat in the milk used increased.

The use of homogenized milk for cottage cheese manufacture resulted in a cheese of excellent flavor and texture quality. The flavor and texture scores increased with the percentage of butterfat in the cheese. The butterfat increased the smoothness of texture and richness of flavor.

#### M43. The Effect of Temperature upon Score Value and Serving Properties of Cheese.\* W. S. ARBUCKLE, J. E. EDMONDSON AND L. E. MULL, Missouri Agricultural Experiment Station.

Recent investigations reveal that temperature has a marked effect upon flavor, body, and serving properties of certain dairy products.

Submerged flavors and a resistant body exist at lower temperatures in ice cream and butter, while full, pronounced, volatile flavors, and a less resistant body are prevalent at higher temperatures.

This study deals with the effect of temperature upon the score value and serving properties of high, medium, and low quality cheese. The samples

\* Paper No. 630 in the Missouri Agricultural Experiment Station Journal Series.

were scored at 40, 50, 60 and 70° F., and it was found that the low and medium quality cheese received a lower flavor score when judged at higher temperatures. The reverse was true for high quality cheese. As the temperature of scoring was changed from 40 to 70° F., the flavor score varied as much as 2.5 points.

The body score changed considerably at the various temperatures depending upon the type of body studied, and the serving properties became more desirable in most cases at the higher temperatures.

**M44. Economic Barriers Affecting the Dairy Industry.** H. A. RUEHE,  
University of Illinois.

During recent years economic barriers affecting the dairy industry have greatly increased. Some of these barriers are the results of laws enacted by Congress, some originate in state, county or municipal legislation, others are directly due to the misuse of legislation created for other purposes, and there are still others that are the result of activities or organized groups carried on for purely selfish motives.

Trade barriers affecting the dairy industry can be classified in four groups:

1. Activities of organized groups.
2. Wage and hour legislation.
3. Misuse of health regulations.
4. Marketing orders operating under the Agricultural Marketing Act of 1937.

There are two types of organized groups whose activities react as barriers to the industry: (1) labor unions, and (2) producer organizations.

In many markets, labor unions control the working personnel in dairy plants. This has a direct effect on students and graduates of dairy institutions. It is gradually becoming more difficult to place men for summer and permanent employment. Furthermore, unions establish wage scales and in many cities they control the methods for marketing milk. These activities in many instances increase the selling prices of milk and cream and, hence, tend to curtail consumption of these products.

Producer organizations in various milk sheds have been effective in curtailing the milk supply by keeping producers out of the market through control of their membership. The health authorities in some cities refuse to inspect the farms of producers not members of certain cooperative groups, and, since the producers must be approved by the inspecting authorities, such producers are eliminated from the benefits of the market.

Wage and hour laws have a direct bearing upon manufacturing and processing costs of plants operating on an interstate basis. Such plants are at a disadvantage when operating in competition with competitors operating on an intrastate basis.

During the last two decades, there has been a progressive evolution of dairy legislation devoted to sanitary requirements. Such legislation has had the support of the dairy industry and the public has benefited greatly by such protection. In the past few years, a misuse of such legislation has created trade barriers which have prevented the free flow of dairy products in commerce.

Various markets are operating under Federal Marketing Orders established under the Agricultural Marketing Act of 1937. The orders, as established in various cities, vary somewhat but their main purpose is to establish milk prices paid to producers. This activity has been effective in establishing high retail prices which in turn has curtailed consumption of dairy products. Some of these orders contain provisions which virtually close the market to new producers, and thus help to limit the supply available to the market.

Dairy educators should study this problem of economic barriers, and they should assist in eliminating such unwholesome factors. Much can be done by encouraging the reciprocal acceptance of equivalent quality standards of inspection by various agencies. Educators must also assist consumers in understanding such economic problems which are affecting the public's economic welfare.

**M45. The Effect of Cocoa upon the Digestibility of Milk Proteins.** L. D. LIPMAN AND W. S. MUELLER, Massachusetts State College.

Whole milk powder plus a commercial brand of Dutch-process cocoa and whole milk powder plus a commercial brand of American-process cocoa, with and without additional cocoa fat, were fed in comparison with whole milk powder in feeding trials with albino rats. The amount of cocoa added to the diets was approximately 16.5 per cent by weight, which is equivalent to approximately 4 per cent on a fluid milk basis. The digestibility of the milk and cocoa proteins was studied.

The rats were able to digest approximately 85, 69, 71, and 71 per cent of the food proteins when rations containing milk powder, milk powder plus Dutch-process cocoa, milk powder plus American-process cocoa plus 2 per cent cocoa fat were fed, respectively. Subjecting these results to mathematical analysis revealed that the digestibility of milk proteins (85.3 per cent) was reduced 7.8 and 6.0 per cent when the ration contained Dutch, and American-process cocoa, respectively. The addition of 2 per cent cocoa fat to the American-process cocoa-milk rations reduced the digestibility of milk proteins by 5.8 per cent.

The proteins of the American-process cocoa were more completely digested (44.5 per cent) than those of Dutch-process (38.1 per cent), when the ration contained cocoa in amount equivalent to 4 per cent by weight on a fluid milk basis, and cocoa was the only source of protein in the diet. The

digestibility of the proteins in the American-process cocoa was found to be only 41.1 per cent when 2 per cent by weight of cocoa fat was added to the ration.

On the basis that the addition of cocoa to whole milk powder (in quantity equivalent to 4 per cent by weight on a fluid milk basis) does not greatly reduce the digestibility of the milk proteins, we may conclude that the amount of cocoa in average commercial chocolate milk (approximately 1 per cent by weight) has no significant adverse effect upon the digestibility of the milk proteins.

**M46. The Acid Hydrolysis of Lactose and the Preparation of Hydrolyzed Lactose Sirup.** G. A. RAMSDELL AND B. H. WEBB, Bureau of Dairy Industry, U. S. Department of Agriculture.

A study has been made of the effect of time and temperature of heating and of acid and sugar concentration upon the hydrolysis of lactose. As the temperature of a lactose-water mixture is raised to 150° C. (54½ lbs. gauge pressure) or higher, decreasing amounts of HCl are required to hydrolyze the sugar. The proportion of hydrolytic products other than glucose and galactose increases as the lactose concentration of the aqueous mixture is raised from 10 per cent to 80 per cent. A 10 per cent lactose solution may be almost completely hydrolyzed to glucose and galactose at 150° C. in the presence of a small quantity of HCl, but when a 60 or 80 per cent lactose-in-water mixture is used a marked destruction of the hexoses accompanies the cleavage of the lactose. Hydrochloric acid has been found to be a satisfactory acid, and the quantity required is so small that the flavor of the finished sirup is not adversely affected when the acid has been neutralized.

The determination of lactose, glucose, and galactose in hydrolyzed lactose sirup is complicated by the presence of optically active decomposition products having a reducing action. However, it is believed that a close approximation of the composition of the sirups has been obtained. The analytical procedure involved determining the reducing action of the sirups before and after destroying the glucose with yeast according to the Somogyi technique. The combined hexoses were determined by the use of a modified Barfoed's solution. From these results the sum of the lactose and other reducing decomposition products were obtained by calculation.

A clear, sweet sirup of pleasing taste containing glucose and galactose with small quantities of lactose and with some hexose decomposition products can be made easily by hydrolyzing lactose with acid. Such a sirup which can be prepared to contain 60 to 63 per cent solids will keep well and is suitable for table use or for the manufacture of various sweet goods.

**M47. Some Properties of Different Combinations of Whey and Other Materials Which Dry Satisfactorily on the Atmospheric Drum Drier.** E. L. JACK AND A. J. WASSON, University of California.

Whey solids have valuable nutritional properties and when they can be

recovered economically they become a supplementary source of income to cheese plants. When whey alone is dried on the double drum atmospheric drier, a gummy mass results that is difficult to remove from the machine and which hardens when cool so that grinding is necessary to put it into useable condition. For the formation of a continuous sheet of dry material it is necessary to add a drying agent to the whey. Various materials have been used, including skimmilk solids, either in liquid or dry form, and cereal products. This study has been concerned with the properties of different combinations of whey and drying agents which yielded a satisfactory sheet when scraped from the drum.

It was found that when liquid skimmilk was used as the drying agent it required about one and one-half parts skimmilk solids to one part whey solids at low acidities to form a satisfactory sheet. This represents about one part milk protein to two parts lactose. As the acidity increases the amount of skimmilk solids required increases also. When condensed skimmilk was used approximately equal parts of skimmilk solids and whey solids in the mixture formed a satisfactory drying combination. Increasing acidity again required that more milk solids be used. Mineral acids gave substantially the same results as developed or added lactic acid. Ground cereal products were also used. Approximately one part cereal product to two parts whey solids gave satisfactory results. Those found to be useable were flour, corn starch, ground oats (sifted), and ground barley (sifted). The amount of cereals required was not much affected by different degrees of acidity. The lactose: nitrogen ratios and the pH relationships have been determined.

#### M48. A More Precise Method for Estimating Fat in the Babcock Test. E. O. HERREID, Vermont Agricultural Experiment Station.

Observations indicated that the fat column in the Babcock test was estimated under a variety of conditions with regard to light, alining of bottles, and type of calipers. An effort was made to standardize such conditions so that the test might be estimated on a comparable basis by technicians. It is believed that the equipment described fulfills these requirements.

The apparatus used in the laboratory of the Vermont Agricultural Experiment Station was invented by the late J. Hortvet, chemist in the Dairy and Food Laboratory of the Department of Agriculture, Saint Paul, Minnesota. This apparatus was described in old catalogue C, pages 470-71 of the Central Scientific Company, and is called a Milk Fat Caliper. This equipment is not manufactured at the present time.

The original apparatus was illuminated by an electric bulb through an etched glass in the center with a mirror strip on each side; the neck of the bottle being read against the illuminated etched glass and the mirror serv-

ing as a guide to assist in leveling the line of vision at the top and bottom of the fat column with the calibrations on the bottle. Another unique feature of this apparatus is that the fat column is estimated by a mechanical device consisting of two pointers, one adjustable to the lower and the other to the upper extremity of the fat column by means of two knobbed screws.

This apparatus as designed by Hortvet was used, but it became evident that the lighting arrangement could be improved. The combination etched glass and mirror was replaced with white, flashed, opalescent glass thus allowing reading the test against an entire white background. The mirror was unnecessary because the present bottles have a marked line three-fourths the circumference of the neck at each per cent mark to aid in leveling the eye straight across the top and the bottom of the fat column. The original pointers on the mechanical measuring device were too blunt to obtain estimations with ease and accuracy, consequently they were replaced with adjustable needle points held in place by screws. Finally a five inch reading glass was attached that magnified the fat column about two and one-half times.

The time required to read the tests is approximately the same as that with hand calipers when one becomes accustomed to this apparatus. Estimations can be made on milk to 0.025 per cent with ease and precision; however, finer calibrations on the test bottles would be advantageous. A bottle that does not stand level is the only difficulty thus far encountered under practical operations. This equipment will be available for examination.

Acknowledgment is gratefully made to Mr. Henry J. Hoffman, Chief Chemist in laboratories of the Department of Agriculture, Saint Paul, Minnesota, for his courtesy in loaning the Milk Fat Caliper.

**M49. The Effect of Specific Gravity and Coefficient of Expansion of Butterfat on the Accuracy of the Babcock Test.** R. JENNESS, Vermont Agricultural Experiment Station.

Measurement of specific gravity and coefficient of expansion of pure butterfat and of fat siphoned from the Babcock test column was undertaken in order to determine their exact values and to furnish a basis for evaluation of the accuracy of the Babcock test. The fact that the neck of the Babcock bottle is calibrated on the basis of the assumption that the specific gravity of fat is 0.9 at the temperature of reading makes specific gravity data essential. Coefficient of expansion makes possible estimation of differences in reading to be expected at different temperatures and calculation of changes in specific gravity as temperature changes.

Samples representing the Jersey and Holstein breeds, a University herd composite, and a composite from a nearby cooperative milk plant were collected at weekly intervals throughout 1939. They were tested by the method prescribed by Vermont Regulations (1936), read in quadruplicate

to the nearest 0.05 per cent, and the fat from 24 or 48 bottles siphoned off. The Mojonnier method was used as a standard of comparison. Samples of pure butterfat from the same sources were prepared by churning, melting, filtering, and drying at 135° C. under 20–25 inches of vacuum for 5 minutes.

Specific gravity at 37.5°/37.5° C. was determined using 10 cc. pycnometers with thermometer stoppers and side capillary overflow tubes. Coefficient of expansion in the ranges 30°–40° C., 40°–50° C., 50°–60° C., and 30°–60° C. was measured in 10 cc. expansion tubes having necks containing 0.5 cc. graduated in 0.01 cc. divisions.

Specific gravity values at 37.5°/37.5° C. fell from 0.9150–0.9200 in the first 6 months to a minimum of 0.9110–0.9120 in October and increased to 0.9130–0.9140 in November and December. Specific gravities of purified fat followed a similar trend but were uniformly lower (0.9090–0.9120).

The coefficient of expansion of Babcock column fat averaged  $75.58 \times 10^{-6}$  and that of pure fat averaged  $78.34 \times 10^{-5}$  in the range 30°–60° C. for the period January to June 1939. This represents a theoretical potential decrease of 0.0030 per cent fat per ° C. decrease in temperature for 4 per cent milk.

The calculated error of reading at 135° F. due to deviation of specific gravity of fat from 0.9 varied from 0.015 to 0.050 per cent fat underreading in the first eight months but became negligible in the last four.

A few determinations of specific gravity of fat from cream test columns showed consistently higher results than similar measurements on fat from milk tests, but again the same tendency to reach minimum values in October was exhibited.

#### M50. Observations on the Distribution of *Pseudomonas fragi*. H. B. MORRISON AND B. W. HAMMER, Kentucky and Iowa Agricultural Experiment Stations.

The frequency with which *Pseudomonas fragi* causes defects in dairy products makes its distribution of importance. Previous investigations have shown that the organism is often present in raw milk and cream and other dairy products from Iowa and surrounding states. In this study it was isolated from 29 of 176 samples of milk delivered during the cool seasons to an Iowa plant. It was also demonstrated in 16 of 40 samples of milk delivered to a Kentucky milk plant in December but was not found in 17 samples delivered to the same plant in June. It was isolated from 6 of 104 swab cultures from churns and other equipment in 40 Iowa dairy plants and also from 3 of 30 creamery water supplies.

Samples of soil, water, feed and bedding and swab cultures from milking utensils, miscellaneous barn equipment, floors, ledges and the cows themselves were investigated. Seventy-one of 137 such samples obtained on Iowa farms during the winter and spring yielded *Ps. fragi* and 37 of 99

samples obtained on Kentucky farms in the winter yielded it, but it was found in only 2 of 49 samples obtained on Kentucky farms in the summer. Studies on barnyard soil from many of the states indicate that *Ps. fragi* is widely distributed geographically.

With *Ps. fragi* present in the soil, it would be found in materials and on equipment which come in contact with, or are contaminated by, the soil, and in this way it could gain entrance to the milk from water, feed, bedding, utensils, miscellaneous barn equipment, floors, ledges and the cows.

**M51. The Serological Integrity of *Streptococcus lactis*.** J. M. SHERMAN,  
KARL L. SMILEY AND CHARLES F. NIVEN, JR., Cornell University.

The serological grouping method of Lancefield (J. Exp. Med., 57: 571, 1933), which has proved of such great value in the differentiation of the hemolytic streptococci into more or less species-specific groups, has not been successfully applied to the non-hemolytic streptococci except in those groups which contain both hemolytic and non-hemolytic varieties, such as serological groups B and D. In Group B (*Streptococcus mastitidis* and its varieties) non-hemolytic strains may be serologically identified quite as satisfactorily as those which are hemolytic. Likewise, in group D (the enterococci) are found a number of closely related biological entities, the non-hemolytic *Streptococcus fecalis* and varieties, and the hemolytic *Streptococcus zymogenes* and varieties.

On the other hand, those non-hemolytic species which are generally loosely designated as "viridans streptococci" (*Streptococcus salivarius*, *Streptococcus bovis*, *Streptococcus equinus*, etc.) have not as yet been shown to contain group- or species-specific antigens (though there are of course a number of serological types within these several species).

It has long been claimed by many bacteriologists that *Streptococcus lactis* is an enterococcus, identical with *Streptococcus fecalis*. In a number of publications from this laboratory it has been shown that *Streptococcus lactis* and *Streptococcus fecalis* may be clearly differentiated on the basis of several physiological tests; we have also shown that *Streptococcus lactis* does not belong to the serological group D, which group includes *Streptococcus fecalis* (Sherman, J. Bact., 35: 81, 1938). These physiological and serological results have now been completely confirmed by other workers (Graham and Bartley, J. Hygiene, 39: 538, 1939).

In further confirmation of the serological as well as physiological integrity of *Streptococcus lactis* we have successfully produced species-specific grouping sera against this organism. Such sera give good precipitin reactions with the extracts of all strains of *Streptococcus lactis* which have been tested; but give no reactions with *Streptococcus fecalis* or other enterococci, nor with representatives of the other serological groups (A to H inclusive), nor with viridans streptococci or other non-hemolytic species outside of the so-called "lactic group" of streptococci.

*Streptococcus cremoris*, a biologically closely related variety, also appears to be serologically closely related to *Streptococcus lactis*. Whether or not these two "lactic" organisms belong to the same serological group is not clear on the basis of our limited data. Most strains of *Streptococcus cremoris* react weakly with anti-lactis group sera. Our few attempts to produce anti-cremoris group sera have failed. This finding of an apparently close serological relationship between *Streptococcus lactis* and *Streptococcus cremoris* appears to be in agreement with the preliminary announcement of work done at the National Institute for Research in Dairying, Reading, England (Annual Report for 1937, p. 37).

### PRODUCTION SECTION

#### P1. Vitamin C for Sterile or Partially Sterile Sires. PAUL H. PHILLIPS AND HENRY A. LARDY, University of Wisconsin.

A series of investigations has been made concerning the effect of the administration of ascorbic acid upon a herd sire. The results indicate to date that (1) the subcutaneous injection of ascorbic acid resulted in the restoration of the fertilizing capacity of certain impotent bulls; (2) potent bull semen normally contained from 1.5–3.5 mg. of ascorbic acid per 100 cc. of fresh semen, values below 1 mg. were associated with impotency, or poor breeding; (3) high ascorbic acid values, 4.0 mg. or more, on the other hand were associated with bulls with an unreliable breeding record; and (4) the ascorbic acid content of fresh semen, freshly drawn blood and longevity of sperm in yolk-buffer provides a fairly accurate estimate of potency or impotency in the bull.

It is apparent that ascorbic acid is intimately involved in the production of virile sperm. The exact nature of its role in this capacity is not known.

#### P2. The Storage of Dairy Bull Spermatozoa.\* H. A. HERMAN AND ERIC W. SWANSON, Missouri Agricultural Experiment Station.

This study is concerned with the storage and preservation of dairy bull semen to be used for artificial breeding. While various dilutors have been proposed and different storage temperatures have been suggested, there still remain many unexplained factors involving the storage of dairy bull semen so as to preserve its fertility. We have attempted to investigate still further the practicability of storing the undiluted semen, collected by means of the artificial vagina, at the usual electric refrigerator temperature of 40° to 50° F. Whenever possible cows have been inseminated with the stored semen and these results correlated with the usual laboratory examinations of the fresh and preserved semen. Practically all studies conducted on bull

\* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station Journal Series No. 631.

semen have indicated considerable differences in the characteristics of the semen produced at the different ejaculates as well as between individual bulls.

Both diluted and undiluted semen have been used in these investigations. The length of time motility persists under various storage conditions has been carefully followed. It must be recognized, however, that motility alone is a poor index to fertility, and often samples evidencing strong motility are ineffective in settling cows with good breeding histories.

Semen samples representing over 300 separate ejaculates collected from 55 dairy bulls have been stored at 40–50° F. with motility ranging from 20 to 80 per cent maintained after 200 hours. Samples vary widely in this respect. Some samples were very low in motility after six hours of storage. In general the diluted semen of high quality showed no marked increase in survival as compared to the undiluted. Samples which tended to lose motility rapidly were apparently benefitted by the use of dilutors, particularly of the egg-yolk-buffer-type, as shown by higher motility and longer survival than undiluted samples of the same ejaculate. The glucose-buffer type of dilutors seemed to exert no beneficial influence. In many cases the undiluted samples showed greater motility after the same storage period. In the use of stored semen, 20 pregnancies were obtained from 35 inseminations using undiluted semen stored from 4 to 196 hours, with an average storage period of about 48 hours. Using diluted semen at the ratio of 1 part semen to 3 parts dilutor, 13 cows have been settled by 24 inseminations.

In general the second ejaculate, unless bulls were being used regularly, has withstood storage better than the first. Wide variations in the storage capacity of different ejaculates from the same bull, as well as from different bulls, have been observed. These results will be summarized in detail.

### P3. Some Observations on the Morphological Variations in the Spermatozoa of Dairy Bulls.\* ERIC W. SWANSON AND H. A. HERMAN, Missouri Agricultural Experiment Station.

In efforts to evaluate the semen, and the reproductive abilities of dairy bulls a critical examination of 300 separate ejaculates of 55 bulls has been made. The breeding efficiency of many of these bulls is available and has been compared with their semen picture. Included in the examination were (1) initial motility, (2) daily motility of semen stored at 40° F. undiluted, (3) pH determination initially and after motility had been less than 50 per cent for three days or more, (4) observations on appearance and consistency of fresh semen, (5) concentration per cubic millimeter, volume of semen, and total number of spermatozoa per ejaculate. Examinations were made of the stained spermatozoa at 1075 $\times$  magnification for determination of morphological abnormalities. Rose Bengal was used for staining purposes. All semen samples were obtained by use of the artificial vagina.

\* Paper No. 632 in the Missouri Agricultural Experiment Station Journal Series.

Wide variations have been observed in the semen collected from the various bulls and there was wide variation in the character of ejaculates obtained from the same bull. It is exceedingly difficult to evaluate the sire through examination of the semen unless several ejaculates collected at various intervals are available.

The percentage of abnormal spermatozoa ranged from 2.1 to 74.8 per cent. All of the bulls of known good breeding efficiency, with the exception of two for which the average was not considered representative of their normal picture, averaged well below 20 per cent abnormal spermatozoa. All of the bulls known to be of poor breeding efficiency produced more than 20 per cent abnormal spermatozoa. Three sires which were practically sterile had more than 60 per cent abnormals, while four known to be of low fertility ranged from 23 to 37 per cent abnormal spermatozoa.

With the exception of semen containing very high percentages of abnormal spermatozoa, 50 per cent or more, there seemed to be no definite correlation between the abnormality count and initial motility or length of survival with good motility. This observation held true for variations in semen from the same bull as well as for that from different bulls.

Morphological variations of the normally ejaculated spermatozoa could not be correlated in any significant manner with concentration, volume, viscosity, or pH of the semen. In cases where it was difficult to obtain the ejaculate, however, an abnormally high pH (7.0 to 7.8) and a high percentage of abnormal spermatozoa of low motility was observed.

Semen from three bulls, examined after a prolonged sexual rest (2 months or more), showed a higher percentage of abnormal spermatozoa than was characteristic for the same sires in regular service. Only after 4 to 6 collections, obtained at 1 to 2 day intervals, did the morphological picture become normal. The increase in abnormalities was largely due to an increase in pyriform heads.

The most common types of abnormal spermatozoa found were tailless, coiled tails of varying degree, and pyriform heads—spermatozoa with tapering or constriction at the posterior portion of the head.

#### P4. Fecundity and Certain Other Characteristics of Fresh and Stored Bovine Semen.\* H. P. DAVIS, G. W. TRIMBERGER AND GRAVERS K. L. UNDERBJERG, University of Nebraska.

During the past four years artificial insemination has been practiced successfully in the University of Nebraska dairy herd. Previously one of us reported that the average number of natural services required per conception was 2.21. This average number was obtained from a study of 1375 conceptions. When the above study was completed about 20 per cent of the cows suffered from trichomoniasis.

\* This study supported by grant in aid by the American Dairy Cattle Club.

The immediate objectives of the use of artificial insemination were to determine whether this method would increase the breeding efficiency over natural service and whether it would serve as a means of control of trichomoniasis. Other objectives were to conduct studies that would increase the information on certain phases of reproduction and to determine certain characteristics of semen.

The semen samples were obtained by massage per rectum of the genital organs or by use of an artificial vagina. In 400 attempts by the massage method 378 semen samples with very active motile spermatozoa were obtained. The average volume was 5.7 cc. and the concentration was 429,000 per mm.<sup>3</sup> The pH value of the semen was usually above 7.00. A total of 107 conceptions resulted from 181 inseminations. The fecundity of the semen obtained by the artificial vagina was likewise studied. A total of 122 conceptions were obtained from 188 inseminations. The above groups include 56 cows which previously had been bred naturally to bulls which were infected with trichomoniasis.

The study of the characteristics of fresh and stored semen and its evaluation in relation to its fecundity revealed certain facts. The characteristics studied included volume, motility, concentration, pH values, morphology of spermatozoa, and fecundity of the semen. A detailed semen analysis by ejaculates of 11 fertile bulls free from disease representing four dairy breeds whose breeding efficiency was supported with pregnancies was included in this study. The mean volume was 4.2 cc.; the motility 74 per cent; the concentration 734,000 spermatozoa per mm.<sup>3</sup>; and the pH value 6.99. For the determination of the relationship between the four factors, the volume, motility, concentration, and the pH value, each of the factors was correlated with each of the other three factors. When the pH was correlated with the volume and the motility there was a highly significant minus correlation, while when correlated with the concentration there was a highly significant plus correlation. Other correlations were only slightly significant. The percentage of atypical spermatozoa in the semen from the 11 fertile bulls was found to be relatively constant, approximately 18 per cent or less. It was established that there was very little difference between the fecundity of the fresh semen samples of the successive ejaculates. Only the first and second ejaculates were studied. There were 45 conceptions from the semen of the first ejaculate requiring 60 inseminations; 28 from the second requiring 35. Inseminations from undiluted semen samples stored from 24 to 99 hours at 35° and 40° F. resulted in seven conceptions from 13 inseminations; samples stored at 50° F. resulted in four conceptions from 15 inseminations.

#### P5. Outlines and Subject Matter in Teaching Dairy Husbandry Courses. E. N. HANSEN, Iowa State College.

A type of outline has been prepared as a helpful guide in teaching such

lecture-laboratory subjects as the selection and judging of dairy cattle. The headings for this outline, one of which is prepared for each course in a sequence, are:

Meeting Number	Topic for Lecture and Discussion	Laboratory Material	Animals to be used.	Reading Assignment	Written Assignment
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The outlines are arranged to cover all meetings of each course. The first is for a freshman course in "Dairy Cattle Problems." In addition to laboratory work in judging, the following lecture and discussion topics are covered: Information on dairying in the state and nation; dairy cattle on general and specialized farms; desired dairy conformation; general score-card for dairy cattle; methods of giving oral and written reasons; selection of dairy cows and herd sires; the use of grades, high grades and purebred cattle; methods and results of Dairy Herd Improvement Associations; a long-time production program and factors influencing the quantity and quality of milk.

Another outline covers a sophomore and junior course, "Breeds of Dairy Cattle." The lectures and discussion center upon the following topics: Type defects and their evaluation in judging; breed score-cards; the showing classification; origin and development of the dairy breeds; characteristics of the major and minor breeds of dairy cattle; families, noted animals, herds and breeders; factors in the selection of a breed; and pedigree study. Considerable use is made of mimeographed material.

In the laboratory, judging work with frequent oral reasons, on rings of four, six, and occasionally more animals, is given. Assignments are made in the compiling of complete pedigrees and the construction of charts showing the influence of noted animals within each breed. Field trips are made to five or six leading dairy farms for observation of methods of management and for practice judging.

#### P6. An Assay Method for Thyrolactin.\* W. W. HEATHMAN AND C. W. TURNER, Missouri Agricultural Experiment Station.

Thyrolactin, a combination of protein and iodine, has been observed to contain considerable physiological activity comparable to that produced by thyroxine. Since some iodinated proteins may show more activity than others, there was need for a simple method of assay of the various compounds produced. It is well known that the administration of thyroxine in excess, due to the high metabolic rate induced, will cause a reduction if not an actual cessation of growth in animals. For our study we have selected day old White Leghorn cockerels because of their availability throughout the year, cheapness, and normal rapid growth rate.

\* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 623.

The chicks are all fed a standard diet ad libitum. The experimental groups receive, in addition, varying amounts of thyrolactin and the body weight determined every other day for a period of about two weeks.

To compare with the results so obtained, other groups are fed equivalent amounts of iodine in the form of KI. As a standard, thyroxine is fed as well as desiccated thyroid tissue.

**P7. Thyrolactin, A New Source of Thyroxine for Dairy Cattle.\*** C. W. TURNER, Missouri Agricultural Experiment Station.

The feeding of desiccated thyroid tissue or the injection of thyroxine has been shown in a series of experiments to cause a rapid rise in the rate of milk secretion, including the percentage content of fat and solids-not-fat. This observation has been of great scientific interest because it has indicated a role of another hormone in regulating the level of milk secretion of dairy cattle. The practical value of this discovery has been nullified very largely by the exorbitant cost of thyroxine and of desiccated thyroid tissue. It now appears possible that this handicap to the practical application of this discovery may soon be removed and that surplus skimmilk may become the agent by which cows can be injected to produce more milk.

For many years it has been known that when iodine is mixed with protein under certain conditions, it becomes chemically united with the protein and free iodine is no longer present. During the past year or two the evidence has become increasingly convincing that protein so treated contains physiological activity comparable to that produced by thyroxine. In fact a paper has appeared in which the claim was made that crystalline thyroxine could be extracted from a preparation of iodinated casein.

Because of our knowledge of the value of thyroxine in stimulating milk secretion and the great practical value of a cheap source of this material, we have begun a study to determine what iodinated proteins will supply the cheapest source of thyroxine activity and whether the cost would be such as to make practical its general use by dairymen. Our preliminary studies indicate that the feeding of small amounts will cause an increase in the heart rate of thyroidectomized and normal goats. It has also increased the milk production of goats. Work with cattle is now in progress.

We have found that fresh skimmilk can be used as the protein. To it is added finely powdered iodine with constant stirring. The casein is then precipitated by adjustment of the pH to its isoelectric point. The iodinated casein is dried and ground ready to be added to the ration.

To this product, the name thyrolactin has been given. The possible advantages of this preparation are listed below:

1. A cheap source of thyroxine activity.

\* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 625.

2. A product of uniform potency when prepared under standard conditions.

3. A material which can be fed as part of the ration.

4. An adequate supply of iodine in its most useful form.

It should be appreciated that this work is in a preliminary state and the only reason for presenting the material at this time is to encourage others to try out our preparations or prepare the material according to our procedure. It will thus be possible to determine in a short time whether its laboratory promise will stand the test of practical application.

**P8. The Effect of Thyroxine Injections on the Physiological Processes of Dairy Cattle.** VICTOR HURST, R. P. REECE AND J. W. BARTLETT, New Jersey Agricultural Experiment Station.

Over a three year period, including all seasons of the year, a series of 11 cows were injected with thyroxine in order to investigate further the thyroid-mammary relationship. Animals in the declining phase of lactation were infected for periods ranging from 5 to 147 days with doses varying from 5 to 25 mg. Synthetic crystalline and the sodium salt, put into solution by different methods, were injected subcutaneously in the shoulder region. Measurements included milk, fat, solids-not-fat, and total solids production, milk color, pulse rates, body weights and rectal temperatures. Results in production varied from negligible increases to rises of 38 per cent in milk production and 59 per cent in fat production. Seasonal variation was found to affect the thyroid-mammary relationship.

**P9. The Ejection of Milk from the Mammary Gland.** FORDYCE ELY AND W. E. PETERSEN, Kentucky and Minnesota Agricultural Experiment Stations.

Eight Jersey cows in the Kentucky Agricultural Experiment Station herd were subjected to a series of experiments to determine the factors involved in the ejection of milk and to what extent the nervous mechanism controls the rate of ejection of milk from the gland. The left half of the udders of three Jersey cows were sympathectomized by removing a two-inch portion of the ilio-inguinal and posterior inguinal nerves as they enter the gland in one trunk at a point immediately below the inguinal ring. These nerves are believed to furnish the only efferent stimuli to the gland tissue, although afferent stimuli are carried from the gland through the ilio-hypogastric nerve.

These three cows and five other cows were subjected to 300 experimental machine milkings to measure the effect of the denervation, fright, intra-jugular injection of adrenalin, and similar injections of posterior lobe fractions on the rate of ejection. The following conclusions seem to be justified.

1. The statement of Dr. Gaines, twenty-five years ago, that the processes of secretion and ejection are separate and distinct, is confirmed.
2. The motor or efferent nerve supply to the secreting tissues serves no direct function in the ejection of milk.
3. Fright causes the prompt cessation of ejection.
4. Intra-jugular injections of adrenalin at the beginning of the milking act causes a similar cessation within thirty seconds. The larger the injection of adrenalin the more time was required before the positive act of ejection was resumed.
5. The intra-jugular injection of posterior lobe fractions (pitocin and pitressin) caused a prompt resumption of ejection within thirty seconds.
6. Some evidence is offered which indicates that naturally-produced pitocin acting upon alveoli and ductule musculature, causing it to contract, is the primary cause of ejection.
7. The theory is advanced that the positive act of ejection of milk is caused by the natural occurrence in the blood of one or more products of the posterior lobe and that the failure to let down milk is similarly caused by an increase in the blood of naturally-produced adrenalin, which probably has the opposite effect in causing the alveoli and ductule musculature to relax. It is believed that the presence of these products in the blood is brought about by afferent stimuli which reach the central nervous system from a variety of sources.

**P10. Effect of Post-Hypophyseal Extract on Lactation in Hypophysectomized Post-Gravid Rats.** ELISEO T. GOMEZ, Bureau of Dairy Industry, U. S. Department of Agriculture.

It was previously reported from this laboratory (*J. DAIRY Sc.*, **22**: 428) that, in addition to anterior pituitary extract plus adrenal cortical extract and glucose, the administration of post-hypophyseal extract (pituitrin) was necessary for the sustenance of young of hypophysectomized post-gravid rats. Since this report was made, additional observations have been accumulated which in general confirm our previous observations.

Injections of 2 to 5 units of pituitrin administered in two equal portions 7 to 8 hours apart, in addition to the anterior lobe and adrenal cortical extracts and glucose, permitted the young to get milk from the mammary glands and as a result of continued treatments young were reared to weaning age (25 days of age). The dosage and frequency of administration of pituitrin seemed to be factors in this phenomenon. When 10 units of pituitrin was administered in two equal portions daily, lactation seemed to be inhibited. On the other hand, while 2.5 units administered in the same manner permit the young to get milk, the same dosage (2.5 units) given in a single injection did not.

The necessity of pituitrin in the secretion and/or excretion (lactation) of milk was further indicated by the fact that withdrawal of pituitrin from the daily régime at any time during the course of the experiment was immediately followed by a rapid loss of body weight of young, terminating in death unless pituitrin injections were promptly resumed.

The average body weights of young rats reared by hypophysectomized mothers treated as above, were very much less than those reared by control animals, the latter including (1) lactating rats subjected to sham hypophyseotomy, (2) normal lactating rats and (3) normal lactating rats maintained on limited daily food intake equivalent to that of the hypophysectomized lactating mother rats. The average daily food intake of hypophysectomized lactating rats was approximately 50 to 60 per cent below that of normals. The daily body weight of hypophysectomized animals, however, was only slightly if at all reduced.

**P11. The Fat Metabolism of the Mammary Gland of the Cow.** J. C. SHAW AND W. E. PETERSEN, University of Minnesota.

In a continuation of the studies of blood fat arteriovenous differences on lactating cows it was found that very little blood fat is taken up by the gland immediately after milking. With the increase of the time interval following milking, blood fat is used in increasing quantities until about four hours after milking, after which time the fat is used in more constant amounts. Calcium presents a similar picture. The use of glucose and amino acids are not so affected. When one half of the udder was milked out and arteriovenous samples were taken from both sides simultaneously it was found that the unmilked side continued to use considerable blood fat, whereas the milked side used little or none. Blood calcium and acid soluble phosphorus were affected in the same direction but were less predictable, especially when the animal exhibited any evidence of disturbance. Blood glucose and amino acids continued to be used in normal amounts on both sides.

The above phenomena were duplicated by the intravenous injection of oxytocin. Large doses of oxytocin prevented the passage of fat into the gland even when the gland was distended with milk. The data indicate that the inhibiting effect of milking upon the use of fat by the gland is due to oxytocin or an oxytocic like principle. The normal passage of blood fat into the secretory cells of the lactating gland and to a lesser extent of calcium and phosphorus is associated with the distention of the alveoli and the secretory cells with milk.

When blood volume changes in the mammary gland were not encountered during the drawing of the blood samples the respiratory quotient was usually in excess of unity. Calculations of the comparative calcium and fat losses to the gland continue to show that the quantity of blood fat used by the

gland is sufficient to account for the milk fat and indicate that very little fat is derived from other sources. In fifty-one experiments in which no blood volume changes occurred in the gland the average fat arteriovenous difference was 9.0 milligram per cent. This difference was confined to either neutral fat and/or cholesterol fractions.

**P12. Some Factors Influencing the Completeness of Milking.** KENNETH MILLER AND W. E. PETERSEN, University of Minnesota.

The effects of the following factors upon the completeness of milking were studied:

1. Lengthening the interval between milking and stripping.
2. Manipulating the udder some time before milking.
3. Lengthening the time involved in milking.

In a study of the effect of the interval between milking and stripping, comparison was made of the milk and fat production of cows stripped immediately after removal of the milking machine and when an interval of 15 minutes was allowed.

The mammary gland was stimulated to "let down" milk by washing the gland 20 minutes before, stripping 15 minutes before, and handling with bare hands 10 minutes before milking. While there was a variation in the response by individual cows, fat production was decreased more than milk production during periods of manipulation. Twelve out of 19 cows decreased fat production significantly; 5 declined over 10 per cent and 4 more than 20 per cent. In milk production, 14 out of the 19 declined; 7 more than 5 per cent, 4 more than 10 per cent, and 2 more than 20 per cent. The variation in both milk and fat production from milking to milking was much greater when the glands were manipulated some time before milking.

To study the effect of the length of time involved in milking in 13 trials, each quarter was milked out separately, requiring about 25 minutes for the milking process. After the last quarter was milked, the milk remaining in the gland was removed following the injection of pitocin. The amount secured before and after pitocin injection formed a basis for calculating the completeness of milking of each quarter. Results showed a decrease in the per cent of the total milk in the quarter in the order of milking. The last quarter milked produced only 75.5 per cent of the milk and 57.9 per cent of the fat produced by the quarter milked first.

**P13. The Effect of Dinitrophenol Administration on Milk and Milk Fat.** G. C. GRAF, L. M. LUDWICK AND W. E. PETERSEN, University of Minnesota.

Dinitrophenol was administered subcutaneously and orally in toxic and non-toxic doses to cows. The toxic doses were continued over a period of two days. The non-toxic doses injected subcutaneously were carried over

a period of five days, and oral administration covered a period of twenty-five days. The non-toxic doses were limited to amounts that would not affect the heart or respiration rates.

With the subcutaneous administration of toxic doses of dinitrophenol, the respiration rate was increased 41 per cent and the pulse rate 55 per cent. A marked decrease in both amounts of milk and butterfat with a marked increase in butterfat percentage resulted. The butterfat composition was altered. The saponification number dropped from 232.2 to 220.2, the iodine number increased from 34.7 to 37.7, and the Reichert-Meisl number increased from 24.4 to 27.4. The milk became yellow from dinitrophenol. Lactose decreased 38.1 per cent; CO<sub>2</sub> increased more than 400 per cent; total nitrogen was unaffected, but casein nitrogen decreased with a corresponding increase in non-protein nitrogen. The increase in CO<sub>2</sub> was due to sodium bicarbonate.

In non-toxic doses dinitrophenol did not affect the amount of milk, but the total amount and per cent of fat was significantly increased. The composition of the fat was but slightly altered in the direction that was noted when toxic doses were administered. Other constituents of the milk remained unaltered.

**P14. The pH of the Bovine Mammary Gland.** PHILIP L. KELLY, Arkansas Agricultural Experiment Station.

Studies are in progress in which thirteen bovine mammary glands have been analyzed for pH. Tissues of six have been studied by means of colorimetric indicators while the remaining seven were studied by means of a potentiometer as well as indicators in some instances.

Studies with colorimetric indicators indicated that the various types of cells present may normally contain a different pH with the connective tissue cells at about 7.0 and the secretory cells ranging from approximately 5.0 to 7.0. Studies with the potentiometer on slices of tissue indicated a pH range from 5.78 to 6.89 for tissue taken from alveolar portions of the gland. The secretory tissues with readings closer to neutrality were non-lactating glands.

**P15. The Hormone Control of Mammary Duct Growth.\*** A. A. LEWIS, Missouri Agricultural Experiment Station.

As a result of work in this laboratory the theory was advanced in 1938 that a previously unrecognized pituitary factor, called mammogen, was the direct agent of mammary growth stimulation. From earlier observations it appeared probable that two fractions were present in the mammogen complex. The duct growth factor (mammogen-I) was believed to be stimulated

\* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 622.

by estrogen alone. The lobule-alveolar growth factor (mammogen-II) was thought to be stimulated by progestin and estrogen during pregnancy.

A study was made of 545 cattle pituitaries to determine the mammogen-I content during growth, pregnancy and lactation. The highest content of this hormone was found in young growing heifers during the first estrous cycles when the mammary duct system is actively developing. The content of mammogen-I found in the pituitaries of pregnant cows was well correlated with mammary gland development during that period reaching a peak at about mid-pregnancy when the growth of the mammary elements is probably most rapid. That lactating cows had more mammogen-I than did dry cows may indicate that the hormone is required to aid in maintaining the functioning gland. The theory that the content of mammogen I in the pituitary is correlated with the estrous cycle was further substantiated in that cows with corpora lutea in the ovaries had considerably more hormone than cows with follicles alone. Furthermore, male rabbits given estrogen had twice the pituitary content of mammogen-I as did normally pregnant does.

It is generally recognized that dairy cows have larger udders and mammary glands than beef cows such as Angus and Hereford. That these differences in size and development are due to genetic factors has long been appreciated but the physiological mechanism by which these inherited differences are expressed has been unknown. This study has shown for the first time that dairy cows exceed beef cows in the rate of secretion of the mammogenic duct growth factor by the pituitary.

Extracts of anterior pituitary containing mammogen-I were shown to develop complete mammary duct systems in male and spayed female mice, rabbits and rats. A large series of hypophysectomized female rats given mammogen-I responded with active proliferation of mammary ducts. Castrate male guinea pigs, which respond to estrogen administration with complete mammary development, showed only duct growth response to mammogen-I extract. Evidently estrogen in this species causes secretion of both mammogenic factors in the pituitary resulting in both duct and lobule-alveolar development whereas direct administration of mammogen-I causes only duct development.

The assay technique for mammary duct growth using the male mouse is as applicable to synthetic mammary growth chemicals as to pituitary tissue and extracts. Assay of several of these pure chemicals showed that estradiol benzoate and stilbestrol were 100 to 240 times as active per unit weight as estrone. Estriol, anol and triphenyl ethylene were about equal in potency at 1/30 to 1/35 the activity of estrone. All previous comparisons between estrogenic chemicals have been on the basis of genital response; vaginal, ovarian or uterine. Such assays were found not to give a reliable estimate of mammary growth potency.

**P16. The Mammogenic Lobule-Alveolar Factor of the Anterior Pituitary.\* JOHN P. MIXNER, Missouri Agricultural Experiment Station.**

Recent studies conducted in these laboratories on the physiology of mammary gland growth have made it necessary to postulate the presence of a second mammogenic hormone which is secreted by the anterior pituitary (AP) and which is directly responsible for the growth of the lobule-alveolar (milk secreting) system of the mammary gland.

White virgin female mice weighing between 10 and 20 grams were used as experimental animals in this study. Such animals have a well developed duct system of the mammary gland, but the lobule-aveolar system which develops only under the influence of pregnancy or pseudo-pregnancy is absent. These animals are ovariectomized and an abdominal mammary gland is taken at the same time as a check on the state of development present in the glands.

Injections of fresh pituitary material obtained from pregnant cattle into these mice caused the proliferation of the lobule-alveolar system of their mammary glands to the condition comparable to four to eight days of pseudo-pregnancy or pregnancy. Such pituitary material has considerable mammogenic duct growth potency as assayed on male mice by the method developed in this laboratory.

An extract of cattle pituitaries has been prepared by extracting the material with warm alcohol and ether. The alcohol and ether is evaporated and a lipid-like material is left. Twenty-five hundredths of a milligram of this material will cause definite duct stimulation in the male mouse which normally has only mammary gland rudiments. This same extract when injected into ovariectomized virgin female mice in amounts ranging from 0.25 mg. to 40 mg. and for injection periods varying from six days to thirty days failed to cause lobule-alveolar stimulation.

A protein-like fraction of the pituitary gland secured by acetone, drying the fresh pituitary caused lobule-alveolar development in these castrate female mice in amounts comparable on a dry basis to the amount of fresh pituitary material required to secure similar development.

It appears then that there are two mammogenic factors of the AP which control mammary gland growth and that a chemical separation of these factors has been effected by the methods of extraction and fractionization used.

As a result of the various experiments it was decided that the female mouse was a suitable assay animal. A mammogenic lobule-alveolar mouse unit is tentatively defined as the amount of material required per mouse, injected over a period of six days, to secure definite lobule-aveolar develop-

\* Contribution from the Dept. of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 624.

ment in  $50 \pm 10$  per cent of 10 or more castrate, nulliparous, female mice weighing between 15 and 20 grams.

Preliminary assays on lots of pregnant and non-pregnant cattle pituitaries have been determined. One hundred and twenty-five milligrams of pregnant cattle pituitary has given a mouse unit, while six hundred milligrams of non-pregnant pituitary failed to give a response. This agrees well with results predicted on the basis of the physiology involved.

**P17. The Effect of Nembutal Anesthesia on the Rate of Milk Secretion, the Respiratory Quotient, and Uptake of Milk Precursors by the Lactating Mammary Gland.\* E. P. REINEKE, Missouri Agricultural Experiment Station.**

The recent reports of Petersen and Shaw seriously question the validity of results obtained in milk secretion studies by comparison of arterial and mammary venous blood samples drawn from normal intact animals, and recommend as a more exact procedure the use of mammary gland perfusions. While this technique appears very attractive from the standpoint of eliminating some of the variables encountered in an intact animal, it is open to the objection that the storage depots of the body are eliminated from the system, and therefore, the blood can hardly be considered as representative of normal arterial blood after it has traversed the mammary gland one or more times. Furthermore, it is not known whether the endocrine factors that are undoubtedly concerned in lactation will function in such a system. It would appear that until more information is obtained on this question the respiratory quotient of a perfused udder is of doubtful significance so far as normal lactation is concerned.

A simple method of eliminating excitement or other psychic factors that might tend to upset the normal level of blood constituents during sampling of the intact animal is to completely anesthetize the animal with nembutal. Contrary to what might be expected, totally anesthetized goats continue to secrete milk of normal composition, at an undiminished rate. Uptake by the mammary gland of the known precursors of milk proceeds at the usual rate, and the respiratory quotient is quite constant.

In a series of arterial and venous samples drawn simultaneously from lactating goats under nembutal anesthesia the average respiratory quotient was 1.09, mean deviation 0.0596. Samples drawn after both the artery and vein had been anesthetized locally with apothesine yielded a mean respiratory quotient of 1.15, mean deviation 0.152.

Normally lactating goats, sampled without anesthesia gave a mean respiratory quotient for the mammary gland of 1.17, mean deviation 0.1895.

\* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series, No. 621.

While the average respiratory quotients as determined by the three procedures were nearly identical, the technique of sampling under nembutal anesthesia eliminates much of the variability encountered with other procedures. These results are in agreement with previous reports from this laboratory that the respiratory quotient of the mammary gland of the lactating goat is above unity, indicating synthesis of a portion of the milk fat from carbohydrate. Comparisons of the ratio of the uptake of glucose plus lactic acid to fat with the ratio of lactose to fat in the milk show that under the conditions of these experiments the fat taken up from the blood is insufficient to account for the milk fat.

Analyses of the carbohydrate portion of the plasma proteins indicate that carbohydrate is taken up by the lactating mammary gland in significant amounts as a portion of a glycoprotein complex, while arterial and venous samples drawn from dry goats show no significant uptake of this complex. This "glycoprotein sugar" if metabolized in the mammary gland could serve as an additional source of carbohydrate either for lactose formation or the synthesis of milk fat.

**P18. A Modification of the Allen Blood Fat Procedure.** J. C. SHAW,  
University of Connecticut.

To increase the accuracy of the method the fat tube is completely immersed in water in a constant temperature bath and the reading of the fat column is made through a glass window. The reading is made by means of a reading microscope mounted on a cathetometer with a vernier scale graduated to 0.1 millimeter. This apparatus is also used in the calibration of the capillary tube. A longer and more slender fat tube with a straight filling neck facilitates the addition and mixing of the reagents. The digestion is carried out at 87.5° C. in a constant temperature water bath and the tubes are centrifuged in a heated centrifuge.

**P19. A Study of Some Methods for the Prediction of Butterfat Percentage in Herds of Ayrshire Cattle.** G. A. BOWLING AND D. N. PUTNAM, West Virginia Agricultural Experiment Station.

This study was undertaken in an effort to determine the transmitting ability of bulls for butterfat tests by an analysis of their pedigrees.

The study included sixty-six Ayrshire sires with five or more tested daughters out of tested dams. A daughter-dam comparison was made for each sire, using only first calf herd tested lactation records not exceeding 305 days in length. No conversion factors either for age or frequency of milking were used.

A three generation pedigree was tabulated for each sire studied, listing the tests of each of the three nearest dams (if tested) and the average tests

of all of the tested progeny of each of the three sires and the three dams in the pedigree.

The following plans were used to determine the "Transmitting Ability" of a sire.

Plan A: Average the tests of the daughters of the sire; the tests of the daughters of the dam, and average the results.

Plan B: Average the tests of the daughters of each of the three nearest sires; the tests of the daughters of each of the three nearest dams, and average the results.

Plan C: Average the tests of the daughters of each of the three nearest sires; the tests of each of the three nearest dams, and average the results.

Plan D: Average the tests of the daughters of each of the three nearest sires, and average the results. The females of the pedigree are not considered.

Plan E: Average the test of the three nearest dams. The males of the pedigree are not considered.

Plan F: Average the average tests of the daughters of the sire with the test of the dam.

Plan G: Average the tests of the daughters of the sire; the tests of the daughters of the maternal grandsire, and average the results.

In each case the "Predicted Average Test" of the daughters of a bull is found by averaging the "Transmitting Ability" with the average test of the cows to which the bull is to be mated.

The results of the study seem to warrant the following conclusions:

1. Although there is no significant difference in the results obtained by the use of any of the plans, excepting Plan E, the plans A, F, B, D, C, G, E have the following respective correlation coefficients: .6621, .5748, .5554, .5509, .5423, .5253, and .4435.

2. The plan involving the average tests of the three nearest dams in a pedigree is the least accurate of the plans studied in measuring the transmitting ability of a bull.

3. When predicting the transmitting ability of a bull it is most desirable to use the tests of the progeny of the animals involved.

The authors wish to acknowledge the cooperation of the Ayrshire Breeders' Association in supplying the data for this study.

**P20. The Use of Cellular Antigens in the Blood of Cattle for Determining Parentage.** L. C. FERGUSON AND M. R. IRWIN, University of Wisconsin.

Numerous antigenic substances have been identified in the red blood cells of cattle by means of antisera prepared by immunizing cows against the blood of other cows. The results of a genetic analysis of each of the nine-

teen antigens studied indicate that the cells of an animal contain a particular antigen only if one or both parents likewise possess it. Furthermore, each of the cellular antigens seems to behave as a unit in inheritance, *i.e.*, each is presumably controlled by a single gene. Nineteen of these substances have been identified and designated as A, B, C, etc. From the genetic evidence available for nine of these, each seemingly represents one member of each of nine pairs of contrasting characters (or of multiple allelic series). Whether the contrasting character or characters of any one or all of these are recessive in nature or are completely expressed in the heterozygote is unknown at present. Although no evidence exists for the interaction of genes in producing these cellular substances, the possibility cannot be ruled out.

On the basic assumption that these antigenic substances are gene-determined, they may be used for the exclusion of parentage. For example, the cells of an animal do not contain antigen "A" if neither parent carries it, so if "A" is present in the blood of an alleged offspring from such a mating, there is evidence of confusion in the records.

In the practical application of this method to "field" cases, a blood sample is required from all of the animals involved, *i.e.*, sire, dam, and offspring. By means of exclusion it is possible, in most cases, to determine the parentage of animals, (1) when two or more calves are mixed before being properly identified with their respective dams; (2) when the sire of a calf is unknown because the dam was served by two or more bulls; and (3) when the validity of the registration of a particular animal is questioned.

**P21. Effects of Inbreeding in Dairy Cattle (Progress Report).** G. E. DICKERSON, Wisconsin Agricultural Experiment Station.

In order to determine the possibilities which lie in the development and utilization of distinct superior lines of dairy cattle, relatively uniform in transmitting ability, we need to know: (1) what the average effect of inbreeding is on growth, conformation, reproduction, and production, (2) how much variability there is between different sires or foundation stocks in ability to withstand inbreeding, and (3) what the effects of heterozygosis are in crosses between lines and what influence the level of homozygosity of the lines crossed has on these heterosis effects. This information is particularly necessary for animals whose average transmitting ability is distinctly superior in outbred matings, and, once obtained, would permit a more dependable evaluation of the breeding methods available for dairy cattle improvement than is now possible.

Data on growth, conformation, reproduction and production have been systematically obtained over a three-year period from three large Holstein herds in which comparisons between inbred and outbred progeny by the

same sire are available. The amount of inbreeding varies from sire-daughter matings with some sires to half or three-quarter sister matings with others. Enough data are now available on birth weight (71 inbred and 100 outbred calves\*) and measurements at 6 months of age (58 inbred and 73 outbred calves\*) to make a preliminary analysis of the effects of inbreeding among the progeny of eight sires.

Calves averaging sixteen per cent inbred (equivalent to about three generations of half-brother sister mating, or two-thirds as much as one generation of sire-daughter mating) averaged nearly ten per cent lighter at birth than non-inbred calves by the same sires, after correction for weight differences due to sex and age of dam. This decline in birth weight held for both sexes and for six of the eight sires. Differences in the inherent size of the dams of the inbred and the outbred calves may explain the heavier inbred calves for two sires. These results show that birth weight is determined to an important degree by the calf's own size inheritance, since the dams of the inbred calves were not inbred animals themselves. Tentatively, it appears that the size difference in favor of the outbreds becomes proportionately smaller rather than larger with growth up to 6 months of age.

**P22. Results of Twenty Years Work on Proving Bulls at the Huntley, Montana, Field Station.** R. R. GRAVES, J. R. DAWSON AND D. V. KOPLAND, Bureau of Dairy Industry, U. S. Department of Agriculture.

About twenty years ago a dairy cattle breeding experiment was started at the Huntley, Montana, Field Station of the Bureau to determine if high levels of production could be fixed and maintained in a dairy herd by the continuous use, for successive generations, of sires that had proved their ability to transmit the factors for high levels of milk and butterfat production. At the present time the 7th successive proved sire is in use. The daughters of these proved sires have been tested for production under uniform conditions, and the sons have been loaned to dairymen in the vicinity of the Huntley Station who are members of dairy herd-improvement associations. Since this work started a total of 126 bulls have been loaned. They have sired 3,058 females. Seventy-three of these bulls now have three or more daughter-dam pairs with records. The 924 daughters of these 73 sires produced an average of 11,178 pounds of milk, 3.65 per cent butterfat and 403 pounds of butterfat, calculated to a mature equivalent basis. The 924 respective dams had an average production of 10,226 pounds of milk, 3.56 per cent butterfat and 364 pounds of butterfat, an increase of 9 and 11 per cent. The daughters of 15 of the 73 sires failed to exceed their dams in milk production, and the daughters of 12 of the 73 sires failed to excel their dams in butterfat production.

\* As of March, 1940.

**P23. Average Useful Life-Span, and Causes of Losses of Dairy Bulls.**

R. B. BECKER AND P. T. DIX ARNOLD, Florida Agricultural Experiment Station.

Data have been accumulated with the cooperation of breeders, breed secretaries, and many of the Colleges of Agriculture of the United States and Canada, concerning the useful life-span of bulls, the owners of which valued them sufficiently that their natural lifetime was completed. Bulls culled because of unsatisfactory progeny, to avoid inbreeding in small herds, or because of having access to a more desirable bull, were not included in any of the tabulations.

The average useful life-span of good proved bulls of four dairy breeds, born prior to 1925, were: for 99 Ayrshires,  $11.19 \pm 2.75$  years; 172 Guernseys,  $10.45 \pm 2.58$  years; 277 Holsteins,  $10.77 \pm 2.66$  years, and for 197 Jerseys,  $11.07 \pm 2.56$  years.

Causes of losses among 1,097 bulls of the same four breeds, based on 126 Ayrshires, 302 Guernseys, 399 Holsteins, and 270 Jersey bulls of all ages, were: sterility, 27.6 per cent; died of undiagnosed causes, 23.3 per cent; old age, 10.3 per cent; accidents, injuries and broken bones, 6.8 per cent; wire, nails, and other foreign bodies, 5.4 per cent; lameness, rheumatism, bad stifles and feet, 4.4 per cent. The total losses from infectious diseases amounted to 12.8 per cent, of which pneumonia and lumpy jaw (actinimycosis) accounted for 2.1 per cent each; tuberculosis, 1.8 per cent; Bang's disease reactors, 1.3 per cent; tumors and abscesses, 1.0 per cent.

Life expectancy tables are being calculated. Further records will be accumulated for use in this study over the next five years.

**P24. The Inheritance of the Solids-Not-Fat Percentage in Dairy Cattle.**

H. C. MOORE AND K. S. MORROW, New Hampshire Experiment Station.

Studies at this station carried on for the last four years on the abnormal relationship (ratio) of fat to solids-not-fat in milk indicate that the cause of variations in solids-not-fat of mixed herd milk from month to month is due largely to the make-up of the milking herd.

The influence of the factor of inheritance upon the solids-not-fat percentage in milk from individual cows was studied, using the method employed by the United States Department of Agriculture in proving bulls for milk and butterfat production. Using only purebred animals, dam and daughter comparisons on the progeny of three Holstein and two Jersey bulls have been completed.

The results tend to indicate that the three factors, milk production, percentage of butterfat, and percentage of solids-not-fat may be inherited separately. A given sire may not affect at all or may decrease or increase

percentage of solids-not-fat, irrespective of changes in percentage of butterfat or total milk production.

One sire increased the solids-not-fat content 0.16 per cent, although showing a decrease in butterfat percentage of 0.21 per cent, with no significant change in milk production. Another sire, used in the same herd, increased the solids-not-fat and butterfat percentage 0.28 per cent and 0.24 per cent respectively, with an accompanying increase in milk production of 242 pounds. A third sire, used in a different herd, lowered the solids-not-fat content 0.11 per cent and increased butterfat percentage 0.06 per cent, and milk yield 428 pounds.

Of the other two sires, one increased milk production 938 pounds and decreased the fat percentage 0.18 per cent without a significant change in solids-not-fat percentage. The other decreased milk production 1118 pounds and at the same time increased both the fat and the solids-not-fat percentage at about the normal relationship of these constituents in milk.

An interesting comparison was available wherein a group of eight daughters of sire B out of paternal sisters (daughters of sire I) all showed an increase in percentage of both solids-not-fat and butterfat. Proportionally, the increase in percentage of solids-not-fat exceeded the expected value in relation to the increase in percentage of butterfat.

**P25. Some Factors Affecting Breeding Efficiency in Dairy Cattle.** R. E. ERB, J. W. WILBUR AND J. H. HILTON, Purdue University.

A study of the breeding efficiency in the Purdue University dairy herd for the twenty-year period (1920-40) revealed considerable seasonal variation. The month of May with 74.3 per cent had the highest average efficiency and the month of August with 58.2 per cent the lowest average efficiency for the year. The twenty-year study included 1,440 services resulting in 922 conceptions. No services were included unless they resulted in calving while the animals were still in the Purdue herd.

The data also show that 72.1 per cent of all conceptions resulted from a single service, with 18.7 per cent from the second service, 6.3 per cent from the third service, 2.3 per cent from the fourth service and 0.65 per cent from over four services.

There was considerable variation in breeding efficiency between sires used in the herd during the period covered in this study. One and two-year-old bulls had the highest breeding efficiency but showed a gradual decline with age thereafter.

An analysis of the prominent cow families in the herd indicated that some families have a higher breeding efficiency than others. This may be an inherited characteristic.

**P26. Early Recognition of the Freemartin Condition in Heifers Twin-Born with Bulls.** W. W. SWETT, C. A. MATTHEWS AND R. R. GRAVES, Bureau of Dairy Industry, U. S. Dept. of Agriculture.

According to definition a freemartin is a sexually imperfect female calf twin-born with a male. Occasionally a heifer born co-twin with a bull is normal. Because of the uncertainty, many breeders follow the practice of destroying all such animals soon after birth. Some breeders, however, are willing to spend the time and expense necessary to raise them to breeding age in the hope that they will be sexually normal.

A study has been made of the conformation, anatomy and udder characteristics of 17 heifers that were born co-twin with bull calves. Fifteen of them proved to be freemartins. The other two apparently were normal although their normality could not be established with certainty because of the fact that they were slaughtered before they reached the age of sexual maturity.

A number of physical characteristics were found to be associated with the freemartin condition. These characteristics should be useful in determining at an early age whether or not any individual female born co-twin with a bull calf is likely to be capable of reproduction. Four characteristics which occurred with high frequency in freemartins were: (1) A retarded development of the mammary gland tissue, (2) an atypical form of the mammary gland tissue, (3) the occurrence of a fold of skin, sometimes containing a cord, extending along the median plane of the body a part or all of the way from the vulva to the navel, which is referred to as a "rudimentary penis," and (4) an enlarged clitoris. In some of the freemartins all of the described characteristics were found. In others only one or two were detected. One or more occurred in every one of the 15 cases that proved on autopsy to be sexually deficient, but none was found in the 2 cases that were found to have normally developed internal genitals at the time of death.

The chances are slight that the heifer twin-born with a bull calf will be sexually normal. In some cases positive determination probably cannot be made until the age of sexual maturity. If one or more of the abnormalities described are present it probably will be good economy to dispose of the animal.

**P27. Some Factors Relating to Bloat in Cattle.** DWIGHT ESPE AND C. Y. CANNON, Iowa State College.

There is little difference in the rate of gas formation between finely cut fresh alfalfa and bluegrass, when placed in rumen fluid and held at  $37\frac{1}{2}$ ° C. Amounts of salt, soda, hydrated lime or combinations of the three which the cow will tolerate in her drinking water increase rather than suppress gas formation. Frosting of alfalfa or bluegrass does not materially change the rate of gas formation.

**P28. Extreme Rarity of Cancerous Growths in the Cow's Udder.** W. W. SWETT, C. A. MATTHEWS AND R. R. GRAVES, Bureau of Dairy Industry, U. S. Dept. of Agriculture.

A study of the anatomy of the udders of more than 400 cows, heifers and freemartins over a period of several years has brought to light lesions of various types. In addition to sizeable abscesses, clefts, and the development of fibrous and scar tissue that usually followed infection or injury, small abscesses, cysts and deposits of various kinds have been found that were not anticipated and for which no plausible explanation can be found in the recorded history of the cow.

It is particularly noteworthy that, in the hundreds of udders examined no growths or tissue changes that appeared to be of a cancerous nature have been found, despite the fact that 31 per cent of the 313 cows of lactating age were over 8 years of age and presumably had reached that period of the life cycle in which mammary cancer may be expected to make its appearance in susceptible species.

These observations are supported by results reported by a number of investigators who have concluded that cancer is virtually non-existent in the bovine mammary gland. In fact, as a result of reviewing the laboratory findings in connection with tumors observed in connection with the meat inspection activities of the Bureau of Animal Industry involving the slaughter of many millions of cattle over a period of years, the conclusion was reached that cancerous growths in the bovine mammary gland are very rare, and that those that were found apparently had originated from carcinoma of the skin and invaded the udder from that source.

In the light of recent studies with mice which show that the breast cancer incidence may be increased in the young of a low cancer strain if they are allowed to nurse females of a high cancer strain, or vice versa, it is gratifying to know that the cow's udder—the source of one of the most important foods used for human consumption—is practically free from cancerous growths.

**P29. Heavy Corn Feeding as a Contributory Factor to the Development of Mastitis.** EARL N. MOORE AND H. O. HENDERSON, West Virginia Agricultural Experiment Station.

In recent years considerable thought has been given to certain contributory factors which might predispose animals to mastitis. The quantity and quality of the ration has been mentioned as a contributory factor.

With this thought in mind a study of the effect of heavy corn feeding as a contributory factor to the development of mastitis has been made. A concentrate ration composed of corn, corn gluten feed and necessary minerals was fed to 14 experimental animals. A control group of 13 cows was fed the

regular herd ration. The digestible crude protein, and total digestible nutrients was approximately the same in both rations. Alfalfa hay, corn silage with pasture in season, constituted the roughage.

The two groups of animals were divided as evenly as possible taking into consideration the age, number of lactations, and previous history of mastitis. Likewise the production level was practically the same between groups, which averaged over 11,000 pounds of milk. Half of the animals were observed for one lactation and the others completed approximately two lactations.

To detect the presence of mastitis the following tests were conducted at intervals of 14 days: (1) Physical examination, (2) Strip cup, (3) Brom thymol blue, (4) Chlorine determination, (5) Hotis test, (6) Leucocyte count, (7) Microscopic examination and (8) Blood agar plates.

An analysis of the results failed to show any appreciable difference in the incidence or severity of mastitis of the two groups. The different test methods used showed fairly good agreement.

**P30. Short-Wave Diathermy Treatment of Bovine Mastitis.\* C. W. MCINTYRE,<sup>†</sup> A. C. RAGSDALE, AND E. R. GARRISON, Missouri Agricultural Experiment Station.**

Short-wave diathermy applications of one hour daily were applied to the udders of cows secreting abnormal milk or with abnormal udder conditions in three purebred dairy herds and of one and one-half hours duration in a fourth herd. The diatherm used had an input power of 275 watts with a 6-meter wave-length and a frequency of 50 megacycles per second. Each cow in the first three herds was treated daily, so far as possible, until a negative test was obtained for all quarters of the udder, or until the termination of the experimental period here reported. In the fourth herd treatments were made for five consecutive days each week and samples of the milk for testing taken on the seventh day.

Positive quarters were determined by the Hotis test, supplemented by the plate count for number of bacteria, a microscopic examination of the incubated milk to determine the type of bacteria, body cell count, chloride test, strip cup or physical examination of the udder. Hotis tests were made on the milk from each of the quarters of the udders of all cows at frequent intervals. Samples showing no color change of any kind were classified as negative. All others were classed as positive or suspicious. Suspicious samples were then examined microscopically.

Ninety-two cows were treated with short-wave diathermy. Thirty-eight of these had been infected for more than 6 months and 54 for less than 6

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† Superintendent of the Hatch Dairy Experiment Station, University of Missouri and Bureau of Dairy Industry, U. S. Department of Agriculture.

months. According to the Hotis test the 38 cows infected longer than 6 months were positive in 101 quarters. Seven or 18.4 per cent of the cows became negative in all quarters after an average of 76 treatments for the group. However, the total number of positive quarters at the beginning of treatments was 101 as compared with 94 at the end of treatments, a decrease of only 7.0 per cent.

The 54 recently infected cows were positive in 81 quarters. Twenty or 46.5 per cent of the 43 cows positive in one or more quarters became negative in all quarters after an average of 41 treatments for the group. The total number of positive quarters at the end of treatment was 41, a decrease of 50.0 per cent.

Combining all herds and groups the 92 cows showed a reduction in the number of quarters positive to the Hotis test of from 182 to 135 or 25.8 per cent during the period covered by diathermy treatment. Shortly after the end of the treatments, however, the number of positive quarters increased to 174 or partially exactly the same number at the beginning of the experiment. Milk produced by all except a few cows was normal in physical condition and appearance at the time of the last treatments, although a number of additional cows again showed abnormal milk on subsequent tests.

The types of bacteria, plate count, body cell count, and per cent chlorides were determined on 35 of the 38 cows infected more than 6 months and 48 of the 54 cows infected less than 6 months. The first tests were made July 5 to 12, 1939, and the second tests September 8 to 14, 1939, but are not available for the dates of the first and last Hotis tests.

The data presented indicates a very definite improvement in the physical condition, appearance and flavor of the milk of cows when treated with diathermy under the conditions of this investigation. This is obviously of great economic importance to the dairymen. The data is equally definite in indicating only temporary improvement, or simply an arrested condition, when results are measured by the Hotis test. There is no evidence of significant change in types of bacteria, plate count, body cell count or per cent chlorides over a two-month interval during the period covered by the tests. Finally the experimental work reported must be considered as preliminary and no final conclusions are drawn by the authors.

**P31. Purified Diet Studies with Calves.** P. E. JOHNSON, J. K. LOOSLI AND L. A. MAYNARD, Cornell University.

The purified diet technique was used to study the growth requirements of dairy calves. The calves were started on the purified diets at two to ten days of age. A mixture of casein, lactalbumin, sugar, butter or lard, minerals and water was fed as a substitute for milk. A dry feed composed of casein, starch, sugar, cottonseed oil, cellophane and minerals was kept before the calves after the first few days, and they were transferred to the dry feed completely after about three months.

The growth rates of the 15 calves studied were below normal in most cases in comparison with Ragsdale's Standards. Poor food consumption associated with periodic digestive upsets seemed to be largely responsible for the slow growth. It was necessary to supply about 25 mg. of magnesium per kilo of body weight to prevent hypomagnesemia, convulsions, paralysis and death. Diets devoid of thiamin or riboflavin gave as good growth as those containing yeast or liver supplements. No improvement in the rate of growth or general well being of the calves was noted when the "grass juice factor" or vitamin C was added to the diet.

**P32. Changes in pH and in Bacterial Count of Milks Sham Fed to a Dairy Calf.** GEORGE H. WISE, G. W. ANDERSON AND J. C. JONES, South Carolina Agricultural Experiment Station.

In order to ascertain the extent to which the hydrogen-ion activity and the bacterial count of milks are altered in passing through the oral and esophageal cavities of the calf, pasteurized whole milk and unpasteurized separated milk were sham fed. As the calf consumed the milk from a nipple feeder, the liquid (milk mixed with various secretory fluids) was collected through a conduit inserted into the distal end of the esophagus, the entrance being made progressively via a ruminal fistula, the rumen and the cardia.

An unconsumed sample (control) and its corresponding sham fed sample were incubated at a temperature of 37° C. for 12 hours, during which time routine bacterial plate counts (on liver infusion agar) and pH determinations were made every two hours. In addition pH measurements were made on the sham fed whole milk at ten-minute intervals during the first hour.

The control pasteurized whole milk during the twelve-hour period gradually increased in bacterial count from 396 per cc. to 68,600,000 and decreased in pH from 6.61 to 6.12. The bacterial count of the corresponding consumed sample immediately after collection was 119,000 per cc., a considerable increase resulting from sham feeding. After collection, the number of bacteria decreased to 22,050 per cc. during the first four hours but slowly increased during the next eight hours to 667,875 per cc., a count markedly less than in the control incubated the same length of time. As the bacterial count decreased, there was an increase in hydrogen-ion activity, the pH dropping to 5.50 by the conclusion of four hours, the rate of decrease being greatest during the first 30 minutes following feeding. The pH showed no perceptible change from the fourth to the tenth hour, but subsequently began decreasing again.

In the control unpasteurized separated milk the bacterial count increased and the pH decreased throughout the entire period, the rate of change being somewhat greater than in the control sample of pasteurized whole milk. The alterations of the consumed separated milk, after the initial increase in bacterial count resulting from sham feeding, were in the same order as, but of a greater magnitude than, in the control.

Rancidity accompanying the rapid decrease in pH of the sham fed whole milk suggested that lipolysis was involved. The indications are that the source of the enzymes is the saliva and/or other fluids in the mouth and esophagus. However, the evidence is not adequate to warrant a final conclusion.

Since the sham fed separated milk manifested no bactericidal properties, some phase or product of the lipolytic reaction evidently was responsible for the lethal effect of the sham fed whole milk medium. Increased hydrogen-ion activity was probably a factor inhibiting multiplication but is not generally considered to be lethal. Lowering of surface tension by fatty acids liberated in the reaction was perhaps a factor preventing the growth of many bacteria. Since differential counts were not made, the relation of the change in bacterial quality to the change in total count cannot be assessed.

**P33. Studies With Barn Air-Cured Alfalfa Hay.** C. E. WYLIE, S. A. HINTON AND J. A. SCHALLER, University of Tennessee and Tennessee Valley Authority.

Continuing studies of curing hay in the barn and feeding value of air-cured hay, investigations were conducted during the summer of 1939 on automatic controls for curing equipment and curing hay to depths greater than 10 feet, which was previously considered a maximum depth.

The barn-curing system consists of air ducts, constructed of lumber on the floor of the hay mow. Partially dried hay from the field is stored as usual in the mow, over the air ducts. An electrically driven blower connected to the air ducts forces air through the hay and removes the moisture.

The most promising method of automatic control for the curing equipment embodies the use of a humidistat and time switch. The humidistat is located outside the barn, and starts or stops the motor according to the relative humidity of the air. The time switch will start or stop the motor for a short operating period at any time desired by the operator. This method required a minimum amount of attention and worked accurately and satisfactorily.

Alfalfa hay was cured without heating or molding to a depth of 10 feet by using the floor ducts. Hay was also cured satisfactorily to a depth of 18 feet by using flexible ducts placed on top of the first 10 feet of hay. Further investigations on curing hay to depths greater than 10 feet are planned for the summer of 1940.

The average power requirement for curing hays of 45 to 50 per cent moisture content to 20 per cent moisture content, using a 5-horse-power motor and a blower delivering 12,000 cubic feet of air per minute, is 43 k.w.h. per ton of dried hay. During each of the winters of 1937-38, 1938-39, and 1939-40, ten yearling heifers from the University dairy herd were fed for 150-day periods. These heifers were divided into two groups as equally as

possible, according to number, breed, age, weight, height, and heart girth. One group of heifers was fed all the air-cured hay that they would consume, while the other was fed field-cured hay ad libitum. Two pounds of grain and ten pounds of corn silage per animal per day were fed to both groups. The heifers in both groups have made normal growth with no marked difference in favor of either.

On all analyses the protein content of the barn-dried has been much higher than the field-cured hay. Samples of the air-cured hay averaged 16.78 per cent protein in 1938-39 and 17.88 per cent in 1939-40. Samples of the field-cured hay have averaged 12.97 per cent protein in 1938-39 and 13.09 per cent in 1939-40.

The result of three years' studies on curing hay in the barn and testing its feeding value have shown that a high quality of hay may be obtained by completing the curing in the barn after partially curing in the field.

**P34. Dried Grapefruit Pulp for Milk Production.** P. T. DIX ARNOLD,  
R. B. BECKER AND W. M. NEAL, Florida Agricultural Experiment  
Station.

During 90-day double-reversible feeding periods in each of three consecutive years, dried grapefruit pulp was compared with dried beet pulp in balanced dairy rations. These were fed to 24 Jersey cows at a level to supply 40 per cent of the T. D. N., replacing one-third of the hay and silage, and a part of the mixed concentrates.

The production of 100 lbs. of milk (Jersey) required the consumption of 106.8 lbs. corn silage, 34.7 lbs. No. 1 alfalfa hay, 42.5 lbs. of dried grapefruit pulp, 9.75 lbs. cottonseed meal (41 per cent) and 9.75 lbs. corn feed meal. Likewise with 47.6 lbs. of dried beet pulp, the cows ate 110.1 lbs. corn silage, 36.2 lbs. alfalfa hay, 9.85 lbs. cottonseed meal and 9.85 lbs. of corn feed meal, while producing 100 lbs. of milk.

Neglecting changes in body weight, 42.5 lbs. of dried grapefruit pulp were equivalent to 45.1 lbs. of dried beet pulp, as used in these trials. Milk yields obtained were slightly to the advantage of the grapefruit pulp, and gains in body weight slightly in favor of dried beet pulp.

It is concluded that dried grapefruit pulp is equal in feeding value to dried beet pulp, when fed as a carbohydrate concentrate in mixed dairy feeds.

**P35. The Value of the Qualitative Color Test in the Study of Ketosis.**  
C. W. DUNCAN AND C. F. HUFFMAN, Michigan Agricultural Experiment Station.

The purpose of this experiment was to study the incidence of ketosis in dairy cattle and to correlate the intensity of the color obtained by the use of a qualitative color test with the actual amount of ketone bodies found in the

urine by the Van Slyke technique. Approximately 1,400 milking cows in state-owned herds were tested for the presence of ketone bodies in the urine. Forty-five per cent of the cows responded to the color test, whereas less than 10 cows actually exhibited clinical symptoms of ketosis.

In recording the results of the qualitative test the intensity of the permanganate color was classified as follows: 1+, faint color; 2+ and 3+, more pronounced color; and 4+, intense color. When the actual quantities of total ketone bodies were determined for each classification, it was found that the mean values indicated a general agreement with the above classification but when each range of values was taken into consideration, no definite correlation could be established.

The acetone and acetoacetic acid fraction and the  $\beta$ -hydroxy-butyric acid fraction of the total ketone bodies were then determined quantitatively. The mean values found for the acetone and acetoacetic acid fraction showed more correlation with the color test than the total ketone bodies. The range of values for each color classification again showed so much over-lapping that it was concluded that the color test ordinarily used by the veterinarian is of doubtful value in estimating the amount of ketone bodies being excreted by the cow or in the severity of ketosis. This conclusion was further verified when it was found that appreciable quantities of ketone bodies, chiefly  $\beta$ -hydroxy-butyric acid, were present in urine samples which gave negative color reactions. These results suggested the possibility that cows may be excreting significant amounts of ketone bodies in the urine normally.

The diurnal variations in the excretion of ketone bodies were determined for eight cows on metabolism trials and it was found that the excretion increased as rumen digestion progressed. The maximum amount of ketone bodies is excreted in the urine at approximately 5-6 hours after feeding. This observation is in agreement with rumen digestion studies in which it was found that the maximum acidity of the rumen occurs at the height of rumen activity.

The qualitative color test, in the absence of visible clinical symptoms, is not a satisfactory indication of the degree of ketosis in dairy cattle because of the lack of a definite quantitative relationship. The results of random sampling may be further invalidated unless the diurnal variation in the excretion of ketone bodies is also taken into consideration.

**P36. Blood Sugar and Carbon Dioxide Combining Power of Plasma in Relation to Ketosis in Dairy Cattle.** J. F. SYKES, C. W. DUNCAN AND C. F. HUFFMAN, Michigan Agricultural Experiment Station.

In a study of ketosis, the total ketones of the blood, blood sugar and carbon dioxide combining power of the plasma have been determined on a large group of mature dairy cattle. Some of these were made at weekly intervals over considerable periods. With one or two exceptions, clinical

symptoms of ketosis were not evident. With increasing degrees of ketosis, the blood sugar values progressively decreased although all these values fell within accepted normal limits. The carbon dioxide combining power of the plasma remained within normal limits at all levels of blood ketones which were encountered in this particular group of cattle and showed no consistent variations which could be correlated with the degree of ketosis.

**P37. The Relationship of Fat Content in the Dairy Ration to Milk and Butterfat Production.** C. F. MONROE AND W. E. KRAUSS, Ohio Agricultural Experiment Station.\*

Practical grain mixtures containing three different levels of fat have been fed to a herd of 90 purebred Holstein cows. These three different fat percentages of 4.9; 3.5; and 2.8 were obtained by using either ground soybeans, expeller, or extracted process soybean oilmeal as the protein supplements. The protein content of the grain mixtures was equalized as nearly as possible. In other respects the grain mixtures were practically identical.

The feeding program called for two similar trials of 160 days. During the first 50 days of each trial all the cows were fed the basal or high-fat mixture, after which they were divided into three groups, each of which received one of the different fat levels for the remainder of the trial, or 110 days.

At the time of writing this abstract one trial has been completed. There are available data from 15 cows on each of the high-fat and low-fat levels and from 10 cows on the medium-fat level. The results of this trial indicate no significant difference in milk or butterfat productions, that could be attributed to the level of fat feeding.

Data covering two 110-day experimental periods and two 50-day preliminary high-fat periods will be presented.

**P38. Alfalfa Hay Cut at 3 Stages of Maturity; Its Yield, Chemical Composition and Feeding Value for Milk Production.** J. R. DAWSON, D. V. KOPLAND AND R. R. GRAVES, Bureau of Dairy Industry, U. S. Department of Agriculture.

For 3 years the bureau carried on an experiment at its Huntley, Montana, field station to compare the yield, chemical composition, and feeding value of alfalfa hay cut at (1) initial-bloom, (2) half-bloom, and (3) full-bloom stages of maturity. Observations were also made on the effect of cutting at the 3 stages on the stand of the alfalfa. The alfalfa was grown under irrigation on 5-acre tracts, was harvested at the particular stage of maturity under practical farm conditions and was later fed as the sole ration to groups of Holstein cows to compare its feeding value. Cutting at the 3 stages had no appreciable effect on the stand. The 3-year average crude

\* In cooperation with the Ohio State Department of Public Welfare and the Central Soya Company, Inc., Fort Wayne, Indiana.

protein content of the cuttings made at the initial- and half-bloom stages was 18.24 per cent as compared to 15.71 per cent for cuttings made at the full-bloom stage. First cuttings of all stages were inferior to later cuttings. The crude fiber content was lower for the initial- and half-bloom stages. The average yields of field-cured hay in pounds per acre were: Initial-bloom, 8938; half-bloom, 8843; and full-bloom, 6940. The average yield of crude protein obtained per acre was 1427 pounds, 1381 pounds, and 997 pounds, respectively, for the 3 stages. The digestion coefficients for crude protein were 77.7 per cent, 77.1 per cent, and 75.4 per cent, respectively, for the 3 stages. The cows fed the initial-bloom hay as their only feed produced an average of 11,099 pounds of milk and 404 pounds of butterfat (calculated to a mature basis). The cows fed half-bloom hay averaged 9763 pounds of milk and 345 pounds of butterfat, and the cows fed full-bloom hay averaged 8981 pounds of milk and 331 pounds of butterfat. There was little difference in the amount of hay consumed of the 3 stages. The nutrients furnished by the hay cut at the initial-bloom stage appeared to be more efficient than the nutrients from the hay cut at the later stages. The comparative costs per ton of the hay cut at the 3 stages were \$3.72 for the initial-bloom, \$3.97 for the half-bloom, and \$4.23 for the full-bloom hay.

**P39. Cystine as a Possible Deficiency in a Ration of Alfalfa Hay for Milk Production.** C. F. HUFFMAN AND C. W. DUNCAN, Michigan Agricultural Experiment Station.

It has long been recognized that total digestible nutrients in roughages are nutritionally inferior to those of concentrates, which is the basis of Kelner's starch equivalents, Armsby's net energy values, and the productive energy values of Fraps'. In a previous report it was shown that cows fed alfalfa hay, bone meal and salt declined in milk production although more total digestible nutrients were supplied than required for maintenance and milk production. When a part of the total digestible nutrients of the hay was replaced with either corn or beet pulp an increase in milk production resulted.

The results of the Oregon workers with rats indicated that when female rats were fed alfalfa protein at a 10 per cent level as the only source of protein, milk production was reduced. The addition of cystine to this diet resulted in increased milk production.

In order to determine the possible deficiency of cystine in a ration of alfalfa alone, four cows which had received alfalfa hay alone for some time were fed cystine as a supplement. One cow was fed 40 gms. of cystine per day for 15 days. This high level of cystine resulted in a marked reduction of appetite and milk production, but a gain in body weight. The other 3 cows were fed 20 gms. per day for a period of 15 days. Milk production was not affected by the addition of cystine, although later when corn replaced alfalfa in isodynamic amounts milk production increased.

**P40. The Feeding Value of Rye Stillage for Dairy Cows.** K. L. TURK  
AND M. H. BERRY, Maryland Agricultural Experiment Station.

Considerable quantities of rye stillage are available for feeding purposes in Maryland and other states. Rye stillage is a product formerly known as distillers' rye slop. This product is produced largely from rye grains with the addition of some rye malt and barley malt. Since little data are available on this product, an experiment was conducted to determine the feeding value of rye stillage for dairy cows.

The whole slop was used in this experiment and was obtained fresh each day from the distillery. The average composition of the stillage obtained from weekly composite samples was as follows: 94.77 per cent water, 0.29 per cent ash, 1.72 per cent protein, 0.21 per cent ether extract, 0.44 per cent crude fiber, and 2.57 per cent nitrogen-free extract.

The feeding experiment was conducted for a twelve weeks' period with twenty cows of the Ayrshire, Guernsey, and Holstein breeds. The continuous system of feeding was employed. The cows were divided into two groups as equally as possible in all essential respects. Both groups of cows received one pound of U. S. No. 2 leafy alfalfa hay and 3 pounds of corn silage for each 100 pounds of liveweight per day. In addition, one group received a good concentrate mixture, containing 16 per cent total protein, in sufficient amounts to meet the requirements of the Morrison Feeding Standards. The other group received the same ration except one-half of the dry matter in the concentrate mixture was replaced by an equivalent amount of dry matter from rye stillage.

No significant difference in milk production in favor of either ration was observed. When the milk production of both groups was equated to an equal energy basis of 4.0 per cent fat, the 10 cows receiving the stillage produced daily an average of 21.03 pounds of milk while the cows receiving the normal ration produced daily an average of 20.11 pounds of milk. It took 19.68 pounds of rye stillage to replace one pound of grain mixture. With a good grain mixture valued at \$30.00 per ton, the value of the stillage was found to be \$1.52 per ton.

Some difficulty was encountered in getting the cows to consume the stillage at the beginning of the experiment. Its palatability was increased by the addition of approximately one-half pint of cane molasses per cow per day until the cows became accustomed to drinking it. Also, the stillage was more palatable when fed at a temperature of approximately 100° F.

Since the amount of stillage fed was substituted for one-half of the dry matter in the concentrate mixture, the amount consumed by each cow varied from week to week for the same cow and for the different cows. One cow, an Ayrshire, consumed an average of 139.7 pounds of stillage per day. On the other hand, one of the lower producing cows consumed only 26.2 pounds of stillage per day. For all cows, there was an average daily consumption of 45.07 pounds of stillage.

There was some difference in the average weights of the cows in favor of those receiving the normal ration. The cows receiving the stillage lost an average of 13.2 pounds per cow during the 12 weeks of the experiment, while those on the normal ration gained 1.8 pounds per cow during the experimental period. Most of this loss was due to three cows that lost considerable flesh before they became accustomed to the stillage. All of the cows that readily consume the stillage maintained their weight satisfactorily and four of them gained weight.

Rye stillage had no deleterious effects on the flavor and odor of the milk in this experiment. The stillage was fed immediately after milking in all cases.

There was no evidence that feeding rye stillage would increase the incidence to mastitis.

**P41. Fermentation Studies on Alfalfa Silage Prepared by the Phosphoric Acid and Molasses Methods.** H. D. McAULIFFE, R. W. STONE AND S. I. BECHDEL, The Pennsylvania State College.

Alfalfa silages, prepared under exactly comparable conditions with various concentrations and mixtures of molasses and phosphoric acid, have been studied with respect to the micro-organisms present and the chemical changes produced. The fodder, a uniform third cutting of alfalfa, was ensiled in six small experimental silos. Serial samples for bacteriological and chemical analyses were taken from various levels by drilling holes through the silo and removing the silage with a soil auger.

Earlier investigations correlated inferior silages with a high pH and a high content of volatile acids. In spite of large numbers of lactobacilli, the amount of lactic acid was small. Serial analyses showed the first stage of the fermentation to be normal with an increase in lactic acid to a relatively high level and a corresponding drop in pH and in fermentable sugar. When the reducing sugar decreased to approximately 1 per cent by dry weight, a second stage of fermentation brought about a lowering in the lactic acid content and an increase in pH. The fate of the lactic acid was suggested by the continued increase in volatile acids; however, there was no apparent change in the bacterial flora during the secondary fermentation.

Although the six silages were apparently normal as a whole, several levels showed the abnormal fermentation previously observed. Differences in pressure at various levels or lack of uniformity in mixing of the molasses and acid with the fodder may explain the abnormal fermentation that occurred in different sections of the silages.

**P42. The Losses Resulting from the Ensiling of Legumes and Grasses with Varying Amounts of Phosphoric Acid.** O. L. LEPARD AND E. S. SAVAGE, Cornell University.

Three experiments were designed to determine the losses and changes accompanying the ensiling, with varying amounts of phosphoric acid, of the following crops: mixed grass, clover and alfalfa; timothy and other grasses; and medium well matured soybeans.

Farm size silos were used and each was filled with a particular crop. The silos were divided into layers separated by waterproof rubber sheets. A definite amount of acid (68 per cent food grade phosphoric acid), varying from 0 to 24 pounds per ton was added to each layer. The losses of the following were determined: total weight, dry matter, crude protein, ether extract, crude fiber, and ash. Other determinations made included temperature changes, pH, and volatile constituents.

The layer method, when the layers are separated by waterproof material, is satisfactory for making experimental divisions in the silo when material low enough in moisture to prevent excessive drainage is used. Relative results may be secured from material which has drained excessively.

Natural moisture is an important factor in the preservation of silage. It should be as high in moisture as possible without allowing drainage. This cannot be stated as a definite percentage as it depends on the type of crop, fineness of cut, packing and depth of material in the silo. Practically, one must develop the art of determining when a crop is at the right stage for ensiling.

The loss of nutrients from silage which drains excessively may be no more than that of a dry silage, because of spoilage and chemical changes, aided by the presence of more air in the dry material.

The addition of water to a dry crop when ensiled is apparently not a desirable practice. The water does not make a homogeneous mixture, but forms channels and runs down through the silage. This may wash out the added preservative.

The pH was not related to the amount of acid added in the case of timothy and other grass silage or of the mixed silage (grass, clover, and alfalfa). There was a definite relationship in the case of the soybeans in that a lower pH resulted from the addition of larger amounts of phosphoric acid.

There was no apparent relationship between the amount of acid added and the dry matter or the individual nutrient losses.

High temperatures did not result in a low moisture silage unless large amounts of air were incorporated in the silage. Temperatures of various lots of silage containing from 50 to 82.5 per cent moisture were determined. In each case the temperature rose from 4 to 10 degrees centigrade above the ensiling temperature, reaching a maximum of from 26 to 38 degrees centigrade in about 18 days. It then declined in relation to the climatic conditions.

**P43. Effect of Depth of Corn in the Silo on Weight of Corn Silage.**  
JOSEPH B. SHEPHERD, Bureau of Dairy Industry, U. S. Department  
of Agriculture.

At the Beltsville Research Center most of the corn is siloed when the ears are slightly dented to well-dented, but before they are fully dented and hard. From 1937 to 1939 data were obtained on the weight of green corn put in 8 silos, on the total weight of corn silage removed from 6 silos, and on the weight per cubic foot at different depths of the corn silage removed from 5 of these silos.

The silos, 14 feet in diameter, were filled to depths of 41 to 45 feet. Twenty-five to forty tons of corn were put in daily. A jointed pipe distributor was used inside the silo. Most of the corn was chopped in  $\frac{1}{4}$ -inch lengths and tramped by one man.

At filling time, an average of 148.2 tons of corn with 71.92 per cent moisture was put in each of 8 silos. The corn averaged 42.37 feet in depth. The calculated weight per cubic foot was 45.45 pounds corn containing 12.74 pounds dry matter.

This weight of corn is 14.5 per cent higher than that calculated by Chase and slightly higher than that calculated by McCalmont for the same diameter of silo and depth of corn. The calculated weight per cubic foot for the different silos ranged from 42.60 to 50.69 pounds corn and 11.83 to 14.16 pounds dry matter. Corn with the highest percentage of dry matter weighed the least but contained the most dry matter per cubic foot. Well eared corn weighed more, and contained more dry matter per cubic foot, than corn that was only fairly well eared.

As the silos were emptied, the silage was carefully removed until the surface was level without bumps or hollows, at approximately two-foot intervals, and the weight of the silage per cubic foot was calculated separately for each section.

The amount of spoiled silage on top varied from 2,180 pounds for silage stored 28 days to 5,760 pounds for silage stored for 556 days. Losses of dry matter ranged from 8.36 to 27.59 per cent per silo, depending largely upon the length of time stored.

Average weights of corn silage per cubic foot at different depths for 4 silos averaging 27.63 per cent dry matter were found to range from 17.7 pounds at one foot to 54.8 pounds at 35 feet, with little or no increase at greater depths.

From the top of the silage to a depth of 30 feet, the settled silage averaged 47.4 pounds per cubic foot, with a dry matter content of 13.1 pounds. This is a 22 per cent greater weight of corn silage than the figure of 39.0 pounds given by Eckles, Reed, and Fitch for the same depth of silage, a difference explainable by the difference in the moisture content.

A table has been prepared showing the total weight of settled silage down

to different depths. From this table the quantity of silage removed and the quantity remaining in the silo can be easily calculated at any time.

**P44. Broomcorn Silage for Dairy Cattle.** K. E. HARSHBARGER AND W. B. NEVENS, University of Illinois.

Two hundred twenty-three thousand acres of broomcorn were grown in 1939 in the six principal producing states of the United States. About one-eighth of this acreage and one-fourth of the harvested crop were grown in Illinois.

As a rule, the brush is the only portion of the crop used and the remaining stalks are plowed under. Farmers seem to hold the opinion that the broomcorn plant is unpalatable and may be poisonous to livestock.

An investigation was conducted (a) to determine the yields of dry matter in the stalk portion of the broom corn plant; (b) to find a suitable method for the preservation of the stalks as silage; and (c) to study the feeding value of the silage. Two varieties commonly grown in Illinois, Black Jap and White Italian, were used.

Beginning on August 9 and at intervals up to September 20, portions of the crop from measured lengths of row were harvested for determinations of yields and for silage.

**Results.** The yield of dry matter in the stalk portion of the broomcorn crop at the usual stage for brush harvest was found to be equal to that in adjoining plots of hybrid corn harvested for silage. Broomcorn stalks ensiled with no treatment produced silage which was either completely spoiled or very low in acidity and in poor condition at the time the silos were opened 8 to 9 months after filling. Treatment with molasses at the rate of 100 pounds to a ton proved effective in the production of silage that had good keeping qualities and fair feeding value. In this investigation, the best stage for ensiling appeared to be at the time the brush is harvested. Chemical analyses show that broomcorn silage is lower in protein and higher in ash and crude fiber than is corn silage.

**P45. Comparison of *Lespedeza sericea* Silage, Alfalfa Silage, and Corn Silage for Dairy Cows.** S. A. HINTON AND C. E. WYLIE, University of Tennessee.

In order to determine the value of *Lespedeza sericea* as a silage crop and to determine its comparative feeding value with that of corn silage and alfalfa silage, two small silos of ten tons capacity each were filled, one with *Lespedeza sericea* and one with alfalfa. Corn was ensiled in a 200 ton concrete silo.

The alfalfa used was first cutting, cut in early bloom stage on May 25, 1939. The alfalfa was raked and loaded as quickly as possible. The moisture content of each load was determined by the Stark toluene method.

The average moisture content of the alfalfa at the silo was 66.1 per cent. This was adjusted to 70 per cent by adding water at the time of filling. The alfalfa was treated with a mixture of 60 pounds of blackstrap molasses and 10 pounds of 80 per cent phosphoric acid to each ton of green material. This mixture of molasses and acid was diluted with an equal part of water to facilitate flowing and applied with a Papée automatic molasses feeder attached to a Blizzard 500 ensilage cutter. The power requirements for cutting, using a 40 h.p., 3 phase motor, was 2.01 k.w.h. per ton.

The *Lespedeza sericea* was first cutting, cut on June 5 and 6 when the plants were from 12 to 15 inches in height. The moisture content was determined and adjusted to 70 per cent. The *sericea* was treated with a mixture of 60 pounds of molasses and 10 pounds of 80 per cent phosphoric acid applied by the same method that was used in the case of the alfalfa. Both the alfalfa silage and *Lespedeza sericea* silage were of excellent quality and were eaten readily by dairy cows during the winter of 1939-40.

In the feeding trials 12 cows in milk were selected. These cows were divided into three groups as equally as possible according to number, breed, age, size of animals, stage of lactation, milk production, and condition at the beginning of the experiment.

Cows in group I received 20 lbs. of corn silage, those in group II, 20 lbs. of *Lespedeza sericea* silage, and those in groups III, 20 lbs. of alfalfa silage. All groups were fed ground alfalfa hay ad libitum, and 10 pounds of a grain mixture to balance with roughage.

The results of the first 120 days of the feeding trials show that all groups maintained normal body weight. The production of all groups has been normal, with groups I and III producing slightly more milk and butterfat than group II. Group I has produced 12,599 pounds milk, 555.5 pounds fat; group II, 11,740 pounds milk, 506.5 pounds fat; group III, 13,277 pounds milk, 566.8 pounds fat. Group I has consumed approximately 400 pounds less hay than has group II or group III.

**P46. Composition and Nutrient Value of Sugarcane as Fresh Forage, Shocked Fodder and Silage.** W. M. NEAL, Florida Agricultural Experiment Station.

Immense yields and diverse adaptability recommend sugarcane as a forage crop for the lower coastal plains. Three methods of feeding are: as a soiling crop, as shocked fodder, and as silage. Digestion coefficients for sugarcane in the three forms were found to be: for crude protein, 20, 00 and 00; for crude fiber, 55, 50 and 53; for nitrogen-free extract, 69, 65 and 45; and, for crude fat, 56, 46 and 41. The digestion trials were conducted with four steers over 20-day experimental periods.

Total digestible nutrient contents on the dry basis were calculated to be: 62.0 per cent for fresh forage, 57.5 per cent for shocked fodder, and

45.5 per cent for silage. Assigning an index value of 100 to the digestible nutrient value of fresh cane, and allowing for silo fermentations and losses in shocking; then, shocked cane has an index of 84, and silage of 62.

**P47. Is Timothy Hay Adequate in Calcium for Optimum Growth of Dairy Heifers?** H. T. CONVERSE, EDWARD A. KANE AND EDWARD B. MEIGS, Bureau of Dairy Industry, U. S. Department of Agriculture.

There is a wide difference in the requirement of calcium for normal growth in dairy cattle as stated by different experiment stations. These different requirements were summarized by Mitchell and McClure in 1937 as more than 0.43 per cent calcium in the total ration for the Massachusetts Station; as more than 0.25 per cent for the West Virginia Station; and as 0.24 per cent or less for the Michigan Station. Both the Massachusetts and West Virginia stations reported better gains in body weight when at least a portion of the hay fed was alfalfa. The gains in weight at the Michigan Station were as large on the timothy ration as on the alfalfa ration.

Since 1935 a number of heifers raised in the nutrition herd for long time feeding experiments have been fed No. 3 timothy hay with or without bone meal as a supplement. The hay and concentrates fed have been described in previous papers. The grain mixture is high in protein to supplement the timothy hay and contains about 0.15 per cent calcium. The timothy hay used was as low in calcium as could be found on the local market, usually ranging from 0.25 to 0.35 per cent calcium and averaged about 0.30 per cent. Bone meal when fed was mixed first at the rate of 3 per cent and later at 6 per cent of the grain mixture, and the feeding of it was started at six months of age, at the end of the milk feeding period. The Holstein heifers received on the average about 2.0 kgs. of grain and 5.0 kgs. of hay. The daily calcium intake for the group without bone meal was about 18 gms. for the Holsteins and about 13 gms. for the Jerseys.

Eighteen heifers have completed the experimental feeding period of one year, from 6 to 18 months of age. Seven of these received the basal ration of grain and timothy hay and seven received the bone meal supplement. Where possible the animals were paired, the calf making the larger gain during the milk feeding period being placed in the bone meal group. Two calves in each group were not well paired.

The group that received the basal ration averaged to gain 419 pounds during the experimental period and the group that received the bone meal supplement averaged to gain 415 pounds during the period. In none of the five cases where the animals were considered well paired at the start of the experiment did the calf that received the calcium supplement gain more than the calf paired with it on the basal ration.

From another experiment, bone ash analyses are available on 2 calves that received grain and timothy hay and on 2 calves that received grain and alfalfa hay. The calves were killed at 12 months of age. There was no significant difference in the percentage of bone ash. The calves that received alfalfa hay had 60.0 and 60.1 per cent of ash in the dry and fat free humeri and the calves that received timothy hay had 60.1 and 59.5 per cent of ash. This fact gives additional evidence that timothy hay fed in liberal amounts supplies sufficient calcium for normal bone development.

**P48. The Effect of Rations Deficient in Phosphorus and Protein on Ovulation, Estrus and Reproduction in Dairy Heifers.** L. S. PALMER, T. W. GULLICKSON, W. L. BOYD, C. P. FITCH AND J. W. NELSON, University of Minnesota.

Low protein is the most striking accompanying characteristic of the low phosphorus rations fed to dairy cattle and other ruminants in the phosphorus deficient region of Minnesota. An experimental study of the effect of this dual deficiency on reproduction and associated physiological phenomena in cattle was begun in December, 1933, and continued until August, 1939. Two heifers, 22 months of age, and nine calves, five to eight months of age, were placed on rations consisting largely of prairie hay (deficient in phosphorus and protein) for periods ranging from 24 to 59 months for the different animals. A small amount of grain mixture was also fed consisting of two or more of the following ingredients: corn, oats, corn gluten meal (low phosphorus) and molasses beet pulp. Except for brief periods or during lactation (involving two animals) the daily intake of digestible crude protein and phosphorus was quite uniform for each animal, the former varying among the different animals from averages of 0.42 to 0.75 pounds and the latter from averages of 4.9 to 7.8 grams. Consumption of total digestible nutrients was also subnormal, due to the deficiencies imposed. The older animals took on an emaciated, unkempt appearance. The calves grew at a subnormal rate; they developed rough coats and were coarse and unthrifty appearing, with overdeveloped skulls and undersized bodies. The condition of protein and phosphorus deficiency was also shown by (a) the development of osteophagia and general pica, (b) low concentration of blood phosphate, (c) low retention of nitrogen and phosphorus and (d) subnormal mineral content of the bones.

Sexual activity and behavior were studied by Drs. Boyd and Fitch by regular and frequent examination of each animal for physical and psychological signs of estrus and menstruation, including rectal examination of the uterus and the determination of ovulation by palpation of the ovaries. It was found that the latter examination made it possible to determine ovulation with great accuracy. First ovulation was much delayed in all of

the animals, especially in the calves which were undersized, but when it began it continued with normal regularity with no instance of retained corpus luteum. Frequently, ovulation occurred without any symptoms of estrus. Obvious estrus was not always accompanied by "bulling." Menstruation in the young heifers occurred much less frequently than estrus.

Eight of the nine calves were tested for breeding efficiency after being on experiment for periods ranging from 14 to 37 months, the corresponding ages being 21 to 42 months. The oldest four animals conceived at first service when 34-42 months old and conception was also normal in the younger animals when regular ovulation became established. Ten normal calves were dropped although two died during very difficult parturition of the youngest two animals which were much undersized. The heifer that was the oldest at the time of its successful first service expelled a small mummified fetus about five weeks before term after repeated failures to induce abortion of the dead fetus; but she conceived again promptly and delivered a normal calf. Two of the other older heifers conceived a second time early in their first lactation and delivered normal second calves.

**P49. The Effect of Avitaminosis A upon Vitamin C in the Bovine.** W. A. KING, P. H. PHILLIPS, M. E. NESBIT, I. W. RUPEL AND G. BOHSTEDT, Departments of Biochemistry and Dairy Husbandry, University of Wisconsin.

The previous report which suggested that growing calves suffering from a vitamin A deficiency develop a lowered blood plasma vitamin C content has been confirmed. In a series of experiments with Holstein calves fed a low vitamin A ration it has been found that a reduced plasma vitamin C occurs shortly after the symptoms of avitaminosis A appear. The subcutaneous injection of crystalline ascorbic acid seemed to alleviate several symptoms associated with the lack of vitamin A. A noticeable improvement in the rough scaly condition of the hair and skin was obtained. In addition there seemed to be an attenuating effect upon retinal hemorrhages.

Calves which received the A-low ration with added crystalline carotene, at the rate of 63.3 micrograms per kilogram of body weight per day, were less thrifty than those receiving only 35.3 micrograms of carotene obtained from alfalfa. Papillary edema occurred in 2 calves which were fed 63.3 micrograms of carotene. These results suggest that the carotene of alfalfa is more readily available to the bovine than crystalline carotene given in oil, and that another factor in addition to vitamin A is involved in the prevention of the papillary edema associated with avitaminosis A in cattle. These experiments seem to indicate that the lack of vitamin C is in part responsible for the condition.

**P50. Vitamin C in the Nutrition of Dairy Cattle.** G. C. WALLIS, South Dakota Agricultural Experiment Station.

In the course of our studies on the role of vitamin D in the adequate nutrition of dairy cattle some observations were made which indicated a possible vitamin C deficiency as a complicating factor. In several instances the teeth were found to be loose and there was considerable hypotrophy and sponginess of the surrounding gum tissue. The incisors showed more looseness than the molars. In one animal the looseness of the incisors was so pronounced that the entire dental pad and teeth could be pushed down like the fingers of the hand. A tooth from another animal was found in the manger.

A study and analysis of the vitamin-D-deficient ration revealed that it was also very low in vitamin C. Although it is generally assumed that vitamin C is not an essential dietary factor for dairy cows it was decided to make a further study of this situation as these cows had been on the deficient ration for a much longer time than has usually been employed for vitamin C studies.

Since June of 1939 indophenol titrations for vitamin C in the milk and blood plasma have been made at least twice monthly on eleven cows. Four of these were from the vitamin-D-deficient (also vitamin-C-deficient) herd. Two others received a ration of alfalfa hay, corn, and oats. The remainder were from the main college dairy herd. Of the latter, some were on pasture during the summer while the others received only the regular herd ration of silage, alfalfa hay, and a grain mix.

Using all the figures available up to and including March, 1940, the cows on the vitamin-D-deficient ration of beet pulp and a grain mix of corn, oats, corn gluten meal, and bone meal, showed an average of 0.369 mgm. of vitamin C per 100 ml. of blood plasma; the cows from the main herd averaged 0.320 mgm.; and the cows on the ration of alfalfa hay, corn, and oats averaged 0.446 mgm. of vitamin C per 100 ml. of blood plasma. Taking the three summer months only, the vitamin-deficient cows showed an average of 0.366 mgm. of vitamin C per 100 ml. of blood plasma, those on pasture averaged 0.249 mgm., and those receiving the regular herd ration averaged 0.320 mgm.

The vitamin C studies on the milk showed that for all samples from the vitamin deficient cows the average content was 1.75 mgm. per 100 ml. of milk. For cows from the regular college herd the average was 1.79 mgm., and for the two cows receiving alfalfa hay, corn and oats it was 2.05 mgm. Figures for the three summer months taken alone show an average of 1.81 mgm. per 100 ml. of milk for the vitamin deficient group, 1.80 mgm. for the cows on pasture, and 1.77 mgm. for the cows receiving the regular herd ration.

As the level of vitamin C in the milk and blood plasma of the vitamin deficient cows was essentially the same as that of the regular herd cows even

during the summer season when some of them were on pasture there seems to be no evidence that they were suffering from a vitamin C deficiency. These observations support the conclusion that dairy cows can synthesize Vitamin C and are therefore, not dependent upon a food source for this factor.

**P51. Blood Plasma Magnesium in Relation to the Vitamin D Deficiency of Mature Dairy Cattle.** G. C. WALLIS, South Dakota Agricultural Experiment Station.

For approximately the last two years determinations of the blood plasma magnesium have been made in connection with our studies on the vitamin D deficiency of dairy cattle. Analyses have been made regularly at monthly intervals and more often when necessary on eight different animals. Two of these animals may be considered essentially normal from the standpoint of herd management and the others belong to the vitamin-D-experimental herd. A total of 136 magnesium analyses have been made. When these figures were averaged by cows the magnesium content per 100 ml. blood plasma was found to be as follows:

Cow 13E—normal herd management .....	3.28	mgm.
" 150 " " "	3.22	"
" 4E—vitamin D deficient diet plus cod liver oil supplement .....	3.25	"
" 6E—Developed vitamin D deficiency during the period of observation .....	3.27	"
" 7E—Dry cow on vitamin D deficient ration throughout. No pronounced vitamin D deficiency symptoms .....	3.58	"
" 8E—Developed severe vitamin D deficiency during period .....	3.99	"
" 417 —Four months on vitamin D deficient ration. No visible symptoms .....	3.08	"
" 12E—Developed vitamin D deficiency .....	3.84	"

The above information reveals no consistent changes which can be correlated with the development of a vitamin D deficiency. Of the three animals which developed a vitamin D deficiency during this period, two of them, 8E and 12E, had the two highest average magnesium figures and the other one, 6E, had one of the lowest figures.

A further study of the trend of the magnesium values for individual animals during this period reveals that these levels of magnesium are typical for the animals concerned and there was no consistent or significant tendency for the values to become either higher or lower as a vitamin D deficiency developed. The general level of plasma magnesium for the animal, during normal periods seemed to continue with considerable uniformity as the vitamin deficiency developed and also during the time of recovery. For instance, the average for 6E during the four months of the severest vitamin D deficiency was 3.35 mgm. as compared with 3.25 mgm. for the whole period. Similarly, 12E showed an average of 4.00 mgm. for the year and 3.81 for the last three months when the vitamin D deficiency was severe.

When she was turned out for sunshine exposure as a source of vitamin D the daily magnesium values averaged by 10-day intervals for the next 30 days were 4.08, 3.58, and 3.68 mgm. for 100 cc. of plasma respectively.

In conclusion it may be said that the levels of magnesium per 100 ml. blood plasma have been found to be slightly higher for these mature dairy cows than is commonly reported for growing animals. The average level of blood magnesium does not seem to vary significantly from normal for mature dairy cows kept for long periods of time under vitamin D deficient conditions even in those cases where the blood calcium and inorganic phosphorus may be decidedly subnormal. Neither is there a decided trend to higher or lower levels as a vitamin D deficiency develops, nor during the period of recovery.

**P52. Vitamin E Potency of Certain Feedstuffs.** L. S. PALMER, J. W. NELSON AND T. W. GULLICKSON (with the assistance of B. B. Migcovsky and W. W. Kielley), University of Minnesota.

Feedstuffs that are naturally deficient in ether soluble substances would be expected to be deficient in vitamin E. Other feedstuffs might be expected to lack E activity because of their nature or the manufacturing process employed even though they contain moderate amounts of ether soluble substances. Knowledge regarding the vitamin E potency of both classes of feedstuffs should help determine how widely the tocopherols are distributed in feedstuffs and should be useful in designing rations for the experimental production of vitamin E deficiency in the bovine species.

The more or less standard biological assay procedure for vitamin E, using rats, was modified in order to secure a satisfactory result when testing feeds expected to be deficient in the vitamin. Each product was tested alone when incorporated in the standard, basal, E-free ration. Some products were tested again in combination with others to determine their additive effect on reproduction. Positive and negative control groups of rats were employed, the former receiving the basal ration plus wheat germ oil of known potency and the latter receiving only the basal ration. Roughages as well as concentrates were tested. With the exception of meat scraps, fish meal, dried brewers' grains, hominy and corn bran, the ether extract of all products was less than three per cent and for some it was less than one per cent.

The following products were found to be sufficiently rich in tocopherols to insure normal reproduction in female rats when fed continuously from weaning to full sexual maturity, incorporated in the basal, E-free ration in concentrations which would be fed to cattle: Phosphorus deficient prairie hay, reed canary grass, wheat straw, rye straw, oat hulls, corn bran, molasses beet pulp, corn gluten meal, blood meal, meat scraps, fish meal, barley, hominy, wheat gluten and black strap molasses. The following products

were found to be too low in tocopherols to insure normal reproduction under these conditions although a measure of reproduction efficiency was obtained if sufficient amounts were consumed: Corncobs, dried brewers' grains and skimmilk powder. The latter two products did not give any reproduction if present as the sole source of vitamin E at less than 20 per cent level in the basal ration during the period of feeding adopted for the assay. The corncob test was made on the material extracted from the cobs by benzene. The following products were found to be either seriously deficient or entirely lacking in tocopherols in our tests: rice straw, corn starch, dried potatoes, polished rice and solvent extracted, dried distillers' grain.

A less complete study was made of the ability of some of the products mentioned to prevent the characteristic testicular degeneration of male rats which occurs when they are reared on E-free diets. The basal E-free ration containing 30 per cent solvent extracted, dried distillers' grains and the same basal ration in which had been incorporated 40 per cent polished rice, 32.5 per cent dried brewers' grains and 15 per cent skimmilk powder failed to prevent such degeneration.

**P53. Carotene Content of Corn Silage.** EDWARD A. KANE, HERBERT G. WISEMAN, LEO A. SHINN AND C. A. CARY, Bureau of Dairy Industry, U. S. Department of Agriculture.

In previous work from this laboratory it was reported that the carotene in corn silage may vary from 1 to 40 mgm. per kg. of wet weight depending upon the condition of the corn plant from which it was made. The average for 21 samples of silage, made from corn cut when the kernels were slightly dented and before they were fully dented and hard—as is the usual practice on the government experimental farm at Beltsville, Md.—was 9.4 mgs.

The silage fed in the dairy herd at Beltsville has been sampled twice daily every day since March 1, 1937. These samples represent as nearly as possible the silage as fed to the cows. They have been preserved in an ice box until composited monthly and analyzed for carotene. The average carotene content of 30 monthly composites from silage kept in concrete silos, made from corn cut as described above, and fed within a year of the time put up, was 13.2 mgs. per kg., wet weight. The monthly composites varied from 4 to 23.7 mgs. The average carotene in the silage fed from March 1 to August 23, 1937, was 7.5 mgs.; Meigs and Converse had one cow that calved during this period that received this silage as her only source of vitamin A. Her calf was weak at birth and died 2 days later. A cow similarly fed, except that the silage contained 13.6 mgs. of carotene per kg. of wet weight, bore a live vigorous calf; whereas another cow receiving silage that for 2 months before she calved contained 6.6 mgs. of carotene, bore a very weak calf that stood up at 14 hours after birth but was accidentally killed at 2 days of age by its mother lying on it. These results suggest that silage put

up and used as above described may or may not supply enough vitamin A for normal reproduction.

The carotene in the corn plant at the time of ensiling has been determined, and also in the silage at intervals for 2 years. The "carotene" appeared to increase somewhat when determined by the usual methods, but this apparent increase was due to an increase in the amount of colored impurity that could be filtered off chromatographically from the real carotene.

**P54. Changes in the Amounts of Carotene and Vitamin A and in the Composition of Milk Fat in Artificially Induced Mastitis.** P. G. MILLER, E. J. LEASE AND G. W. ANDERSON, South Carolina Agricultural Experiment Station.

A case of mastitis was induced in the left quarters of a young Guernsey cow by injections of a suspension of *Str. zooepidemicus*.\* Determinations of carotene, vitamin A, refractive index, iodine number, saponification number, and Reichert-Meissl number were made on the churned and filtered butterfat from the entire milkings individually collected by quarters. These determinations were made before infection, during mastitis, during sulfanilamide therapy, and for several weeks after treatment.

Before infection the milk produced on the right and left sides of the udder was essentially the same in all properties studied.

During the case of induced mastitis the milk from the infected quarters (left side) changed markedly as follows: the carotene and vitamin A in the fat more than doubled; the amount of carotene per liter of milk almost doubled; the fat content of the milk decreased; the total amount of carotene secreted in the milk per day decreased rapidly and the milk yield decreased to a low level. During the same period, milk from the normal quarters (right side) did not change very much, although a slight increase in carotene per liter of milk and per day occurred. This increase in carotene was probably due to the slight decrease in milk yield of which the fat content was higher. The milk fat from the infected quarters had a lower refractive index, iodine number and Reichert-Meissl number and a higher saponification number than the milk fat from the normal quarters.

Sulfanilamide therapy sufficient to eliminate all hemolytic organisms from the udder did not significantly affect the fat test or milk yield, but markedly decreased the carotene content of the milk fat from both the infected and normal quarters.

Upon recovery from the symptoms of active mastitis, all the various properties studied tended to return to normal, however, the milk yield and fat test remained low and the carotene of the fat remained slightly high from the quarters that had been infected.

\* Isolated and identified from a case of bovine mastitis by Dr. F. B. Hadley, Division of Veterinary Science, University of Wisconsin, Madison, Wisconsin.

Histological examination of the udder tissues, soon after collecting the last samples, showed the infected quarters to be very low in active secretory tissues and to have a large increase in leucocytes and connective tissue.

**P55. The Effects of Vitamin A Deficiency on the Young Male Bovine.**

T. S. SUTTON, W. E. KRAUSS AND S. L. HANSARD, Ohio Agricultural Experiment Station and Ohio State University.

Male calves were maintained on a ration low in vitamin A until about one year of age. These calves were paired with others of the same age, sex and breed for controls. When slaughtered, tissues were obtained for microscopic examination and assay. The following changes were noted in substantiation of the reports of Moore and others: constriction of the optic nerve, partial closure of the optic foramen, papillary edema, low vitamin A content of liver, low blood carotene and kidney degeneration. In addition the following changes were noted: Degeneration of the germinal epithelium of the testes, absence of spermatozoa in the epididymus, and an accumulation of fluid in the cleft between the anterior and posterior lobes of the pituitary. A microscopic examination of the anterior pituitary (one case) showed evidence of an extension of the Alpha cell area. An assay of the anterior lobe for gonadotropic hormone gave indications of an increase in gonadotropic activity. These pituitary changes are comparable to those previously reported in the rat and are believed to represent compensatory activity on the part of the pituitary in response to the dietary damage to the testes.

The practical significance of high vitamin A intake was demonstrated in a trial involving 86 male calves to be raised for veals. When a milk fat substitute-skimmed milk combination was fortified with vitamin A the incidence of pneumonia dropped from 46.2 per cent to 12.5 per cent. This latter incidence rate was comparable to that obtaining in calves fed whole milk and was correlated with the amount of vitamin A found in the liver.

**P56. Cerebrospinal Fluid Pressure and Vitamin A Deficiency.** L. A.

MOORE AND J. F. SYKES, Michigan Agricultural Experiment Station.

In previous publications a type of blindness has been reported resulting from a constriction of the optic nerve. This blindness was found to be due to vitamin A deficiency. The blindness was preceded by papilledema, nyctalopia, incoordination, syncope and a decrease in the carotene content of the blood plasma. The presence of papilledema is usually considered *prima facie* evidence of an elevated cerebrospinal fluid pressure and this together with some of the other symptoms suggested that elevated pressures might accompany vitamin A deficiency in the bovine.

Therefore, the cerebrospinal fluid pressure was measured on young bovine fed a vitamin A deficient ration. The pressure was determined by

cisternal puncture through the dorsal opening of the atlanto-occipital articulation with the animal in the standing position, using a water manometer.

The results showed that a deficiency of vitamin A in the ration of the bovine permitted the pressure to rise from a normal of 90-120 mm. of saline up to as high as 300 mm. This increase in pressure was accompanied by lowered plasma carotene values, nyctalopia and papilledema. That these changes were definitely due to a deficiency of vitamin A was demonstrated by the fact that when crystalline carotene dissolved in cottonseed oil was the source of vitamin A and was withdrawn from the ration an elevation of pressure resulted which returned to normal when the carotene was again returned to the ration. The decline in pressure to normal was usually quite slow which confirms previous observations that the papilledema is slow to recede.

Preliminary results with dogs likewise indicate an elevated cerebrospinal fluid pressure in vitamin A deficiency although the eye changes are not so pronounced.

**P57. The Effect of Carotene Consumption on the Milk Yield of Jersey Cows.** O. C. COPELAND, Texas Agricultural Experiment Station.

Experiments have been conducted to ascertain the effect on milk yield of an inadequate supply of carotene or vitamin A in the ration of recently fresh and high producing Jersey cows. The amount of carotene supplied to one group of cows was 1,500 micrograms of crude carotene per 100 pounds liveweight daily, a quantity comparable to that supplied the average dairy cow in the Southwest during periods of drouth or during periods of feeding without pasturage. The other group was fed a daily allowance of 15,000 micrograms of carotene per 100 pounds liveweight, or an amount more nearly adequate with regards to supplying the carotene requirements for milk production than is commonly furnished dairy herds of this section during periods of drouth, or without green pasturage.

The results of two experiments using twelve cows in each experiment indicate that the milk yield of high producing dairy cows can be reduced through an inadequate supply of carotene or vitamin A over relatively short periods of time, especially during that stage of the lactation period when milk production is at the peak. Cows on the higher level of carotene feeding produced approximately ten per cent more milk than the cows fed on the lower level of carotene.

**P58. The Vitamin A Requirements of Dairy Cows for the Production of Butter of High Vitamin A Value. II. Relative Efficiency of Carotene (Dehydrated Alfalfa Hay) and Vitamin A.** J. W. WILBUR, J. H. HILTON AND S. M. HAUGE, Purdue University.

In these experiments, the criterion for the measurement of the vitamin A

requirements of dairy cows for the secretion of milk fat with maximum vitamin A value is based upon the supposition that cows are not able to secrete butterfat of maximum vitamin A value until the optimum requirements for maintenance and production have been satisfied. Since the vitamin A value of butterfat secreted by the cow is dependent on the ration fed the cow, it is apparent that whenever cows secrete butterfat of low vitamin A value, this is indication of an inadequate supply of available vitamin A in the ration. Furthermore, if more potent butterfat is produced upon increasing the vitamin A intake, this would also indicate that the vitamin supply had been inadequate. Only when further additions to the rations give no further response in the potency of the butter, is there any assurance that a point of saturation has been reached. Thus, the minimum vitamin A potency of the ration which will produce the maximum effect upon the milk fat secreted should prove to be the minimum vitamin A requirement of the cow for the secretion of milk fat of high vitamin A value.

The procedure in these tests was to reduce the vitamin A activity of the milk fat secreted by dairy cows to a low level by feeding vitamin A deficient rations. Then in successive feeding periods, definite quantities of vitamin A potency were introduced into the rations and the effect on the milk fat was determined. When dehydrated alfalfa hay was used as a source of vitamin A (carotene) the cows required approximately 550,000 Sherman-Munsel units daily to restore the vitamin A potency of the butterfat to its highest value. With vitamin A (*per se*) from 100,000-200,000 units in the ration daily were sufficient to effect a maximum vitamin A potency of the milk fat.

#### EXTENSION SECTION

##### E1. The Nation-Wide D.H.I.A. Proved-Sire Program. J. F. KENDRICK, Bureau of Dairy Industry.

The objective of the nation-wide dairy herd-improvement association proved-sire program is to improve the inherent producing capacity of the nation's dairy herds, that dairymen may produce milk more efficiently and profitably.

Association herds, which now have a yearly average butterfat production of 317 pounds per cow, may be further improved. The animals in these herds that have an inheritance for a high level of production may be located and their influence may be perpetuated and disseminated throughout the general dairy cow population of the nation, which has a yearly average butterfat production of only 170 pounds per cow. The 28,000 association herds may serve as a national breeding herd to supply improved breeding stock to our national dairy herd of 25,000,000 cows.

The nation-wide dairy herd-improvement association sire-proving program represents the broadest, most comprehensive, dairy-cattle improvement program ever to operate in this or any other country. Briefly, the plans call

for the eartag identification of all non-registered animals in association herds so that a genealogical record may be established and maintained for all animals in association herds. As the 305-day lactation records of each cow are reported, they are permanently recorded so that dam-and-daughter comparisons will accumulate for every sire used in association herds.

At the present time approximately 80 per cent of the dairy-herd-improvement associations of the country are cooperating on the program. Association testers are now reporting identification and production records at the rate of about 1,300 per day. Up to April 1, 1940, production records had been recorded for approximately 300,000 association cows. Dam-and-daughter data are now accumulating on about 50,000 sires that have been used in association herds. More than 5,000 sires have already been proved. As the program goes into full operation, dairymen and dairy leaders are being provided with information which will enable them to improve the inherent producing capacity of dairy herds.

## E2. The Importance of Selective Registration to the Dairy Industry.

LYNN COPELAND, American Jersey Cattle Club, New York City,  
N. Y.

Systems of selective registration have been followed for years in Holland, Denmark, and on the Channel Islands. It is recognized that these systems have been important factors in the improvement of the dairy cattle in these countries. Even in America selective registration is not entirely new for several breeds of livestock have followed selective registration based on color markings. However, no system of selective registration for dairy cattle based on production has ever been adopted in the past by dairy cattle Breed Associations in America.

The American Jersey Cattle Club in June, 1939, established a system of selective registration for Jerseys applicable to males alone and effective January 1st, 1942. After that date to be eligible for registration, a bull calf must meet certain requirements regarding the production of his immediate ancestry. A bull calf may be registered if the dam has completed a production record meeting certain requirements. If the dam has no record, the calf may be registered if sired by a good proved bull. If this qualification is not met, a calf may be registered, if sired by a bull that has sufficient proved production in his pedigree to qualify for one of the Star awards of the American Jersey Cattle Club.

Approximately ten-thousand new registered Jersey bulls are required annually to meet the demand of the breed. However, these bulls are bred by about 4000 of the approximately forty-thousand breeders of registered Jerseys in milk today. The new requirements should not adversely affect any Jersey breeder and it is hoped that the new program of selective registration will help give more recognition to Registration Certificates in the future.

**E3. Utilization of Proved Sires and Sons of Proved Sires. FLOYD ARNOLD, Iowa State College.**

The national program of identification and the reporting of production records to the U. S. Bureau of Dairy Industry, has greatly speeded up the program of proving sires throughout the United States. In Iowa for example during January, February and March records were received for 134 bulls. This was more than for any full year prior to 1939. The 1940 list of sires proved in Dairy Herd-Improvement Associations throughout the United States contains the names and records of more than 3,000 sires. These were tabulated in the 12 months preceding April 1, 1940.

It is not enough, however, to just compile proved sire records. Plans for keeping promising young bulls in service until proved and for extending or expanding the use of the good proved bulls should be developed and carried out. At the present time less than one sire in four—23 per cent—is alive when proved and of the living proved sires less than half—40 per cent—have shown a significant increase—25 pounds—in the production of their daughters over their dams. Sixty per cent of the living proved sires have maintained or increased the production through their daughters but only 45 per cent had daughters averaging over 400 pounds butterfat.

It is evident, therefore, that only about 1 sire in 8 is alive when proved and, as indicated by the production of his daughters, worthy of further use. The number is further reduced in practice because not all of the bulls that transmit satisfactory production do the same for type and are eliminated on this account.

The possibility of extending or expanding the use of proved sires is further complicated by the fact that by the time they are proved, their years of usefulness are numbered. A few sires—2 per cent—are proved before 6 years of age but an even greater number—3 per cent—are over 12 years. Sixty per cent of the bulls proved are between the ages of 7 and 9 years. The average age of the sires alive when proved is 8 years. According to Lush and Lacy the life expectancy of 8 year old bulls is 2.2 years. For 6-year-old bulls it is 2.7 years and for 10-year-old bulls it is 2 years.

An indirect way of extending the use of proved sires is through their sons and in this way probably more can be done than in any other. Observations made in Iowa show that Dairy Herd-Improvement Association members consider this a real possibility. The number of sons of proved sires in service grew from 93 in 1935 to 222 in 1938. The number in service in 1938 was 14 per cent of the total number. Many grandsons of proved sires (312) were also in service. Evidence indicating that sons of the better proved sires are most in demand was also noted in Iowa. Forty-seven proved sires with sons in service had 532 daughters averaging 438 pounds butterfat and they were from dams averaging 424 pounds butterfat. This is considerably above the average.

It is evident from the foregoing that there is little chance of increasing the use of proved sires except through their sons or programs designed to prove bulls at an earlier age and to keep them in service until proved. Bull studs, bull associations and artificial breeding societies offer the greatest possibilities. Much can be done also through bull record-book projects, better sire contests, sire exchange and meetings at which sire problems are discussed.

**E5. Observations in the Care and Management of Dairy Bulls.** R. R. WELCH, Pennsylvania State College.

The increasing interest in proved sire system of breeding calls for a greater knowledge of proper feed and care and management of dairy bulls that will assure a long life and satisfactory service of the superior sire.

Freedom and exercise of the bull is perhaps of greatest importance. Some have resorted to the use of mechanical means for exercising the bull. Such methods are not practical for the farmer breeder. Large exercise yards that furnish pasture in summer and year around day and night freedom of the bull is proving most satisfactory in Pennsylvania. Bull pens should be long and narrow.

There is a lack of knowledge, based on experimentation, on feeding the bull.

Why do some bulls fail to breed while other bulls kept under similar conditions continue satisfactory service for many years?

Why do some bulls fail to breed after being moved while others do not?

Some bulls become temporarily sterile, and later become satisfactory in service—why?

**E8. Suggestions for Making Better Use of D.H.I.A. Feed Records.**  
R. G. CONNELLY, Virginia Polytechnic Institute.

Dairy herd improvement association data may be divided into four classes: first, those vital data that establish the identity of the cattle; second, those data that indicate the milk and butterfat production; third, those data that refer to the type, quantity, and quality of feed; fourth, those financial data that establish the value of the milk in relation to the value of the feed. Taken in their entirety, these data furnish a practical basis of sound dairy herd management and improvement.

When these data are used, extension dairymen place varying degrees of emphasis upon the four classes. The present tendency is to give special attention to the cattle identification and production data as a basis for measuring the inherent milk-producing qualities of related cattle. Less emphasis seems to be placed upon the feed and financial data, giving to the dairy herd improvement association program the semblance of unbalance due to over-specialization in one direction.

The proving of bulls, the identification of brood-cow families, and the general marshalling of genetic facts pertaining to the inheritance of milk-producing ability in cattle have created a great and encouraging change in the conception of practical dairy cattle breeding. The economics of a balanced program of dairy herd improvement and dairy farm management, however, suggest the need for attention to all classes of dairy herd improvement association data in order to establish the real environmental values under which efficient herds must be bred, dairy farms must be operated, and net profits must ultimately be determined. There seems to be a prevailing need for more exact and better standardized methods for collecting, analyzing, and utilizing, feed, production cost, and other types of dairy herd improvement association data that may contribute to a better understanding of the many interrelated factors that contribute to successful dairy farming.

Experience with dairy herd improvement association records in Virginia suggest: First, that the feed, financial, and production data, can be assembled with a degree of accuracy and completeness that will permit a detailed analysis of each association's herd books at the end of the record year, thereby furnishing a basis for constructive adjustments in the management of the herds and farms of the members.

Second, that when correlated with the production records, the feed and cost data may provide a rather accurate basis for measuring trends in production, efficiency, and in gauging the effects of changes in operating methods in the herd and on the farm.

Third, that the data pertaining to feed consumption, feed costs, and fluid milk values can be assembled to establish a fair basis of appraisement when indemnities are determined for cows reacting to the Bang's disease and tuberculosis tests. The same data may be used when it is necessary to determine the earning or collateral value of the herd when establishing a basis for financial credit.

Fourth, that the data pertaining to feed consumption, feed costs, and fluid milk values may be used with supplemental survey data as dependable testimony before a state milk commission in determining a fair price for fluid milk on the farm.

#### E9. Accuracy and Use of D.H.I.A. Feed Records. C. G. CUSHMAN, Clemson Agricultural College.

It is axiomatic that if feed records obtained through Dairy Herd Improvement Association work are to be of any value to the participating member or of any safe value in extension teaching they must be correct within reasonable limits of human error and judgment. It is to be expected that testers will make mistakes both figurative and mistakes of judgment in view of the facilities available to them on the farm for making accurate computations. There are three general types of error. First, the error in and

tendency to make estimates which can vary widely from fact; secondly, errors in computations which resolve themselves into costly omissions and pure mistakes in simple arithmetic; and third, errors due to a natural leaning of the tester toward the conservative side in his eagerness to please his member with commendable results.

A system of analysis of monthly reports which entail auditing of the monthly reports at the state office and which results in a running chart will show to a trained clerical worker a great many errors. This basic work gives opportunity for annual analyses of yearly reports which develop a great mass of superior educational material. There is no educational material more powerful than accurate and dependable records developed by farmers themselves. Such records cover a wide range of conditions which are, and always will be, met by farmers and which Experiment Stations cannot hope to cover. Thus Experiment Station material, which forms the basis for improvement in practices, must depend upon accurate farm records to adjust Experiment Station results to varying farm conditions. Superior germ plasm in dairy cattle can be of advantage to the dairy farmer only if his management practices are sufficiently expert to make the most economical use of it.

Accuracy in D.H.I.A. feed records, therefore, plus full and adequate use of the lessons these records can reveal become of paramount importance.

**E10. A Method for Determining Feeding Levels in Dairy Herd Improvement Association Herds.** W. T. CRANDALL, Cornell University.

At the end of the dairy herd improvement association record year it is important to know whether the production of the cows in herds is really representative of their true inherent ability. Cows supplied with amounts of nutrients below their needs will either produce on a level with those nutrients rather than on the level of their ability, or run down badly in physical condition. Cows fed more nutrients than are theoretically needed for maintenance and the work they do are likely to either produce inefficiently or to put on excessive body weight, particularly if heavy rates of grain have been fed.

The same method as outlined for use in determining feeding levels during a yearly period may be used to check on the adequacy of feeding methods for any one month.

**INFORMATION NECESSARY FOR DETERMINATION OF FEEDING LEVELS**

In order to determine the feeding level of a herd for a yearly period the following information is needed on the average of all the cows in the herd:

1. Live weight at start of year to determine the T.D.N. required for maintenance.
2. Physical condition at start and close of year to indicate loss or gain during the year.

3. Yearly milk production, and butterfat test to determine the T.D.N. required for production.

4. Amount and quality of all feeds fed during the year to determine the T.D.N. supplied for maintenance and production.

The Morrison Feeding Standard for dairy cows is used in determining T.D.N. requirements. In determining the T.D.N. supplied by feeds, the standard average analyses are used for grain and for succulent roughages, but dry roughages are figured on an estimated productive nutrient basis.

#### DETERMINING PASTURE YIELDS

In order to get a worth while estimate of the pasture yield of nutrients, a table giving the probable daily nutrient consumption of cows of varying weights on pastures of different qualities is used. Dairy herd improvement association testers report the quality of pasture each month as excellent, good, fair or poor and a weighted average is made for the season.

#### RATING THE FEEDING LEVEL OF A HERD

The rating on the feeding level of a herd is made after taking the following into consideration.

1. The amount of plus or minus T.D.N. supplied in feed as compared to those required for maintenance and milk production.
2. The rate of grain fed to milk produced.
3. The physical condition of the herd at the end of the year as compared with their physical condition at the start of the year.

#### A STANDARD OF GOOD FEEDING

A herd has been well fed when on the average the cows in the herd are in good physical condition at freshening time, maintain normal milk curves and fair physical condition during a ten months' milking period and regain good physical condition again before freshening.

##### *Heavy feeding.*

1. T.D.N. supplied are 1000 pounds or more over needs.

2. Rate of grain to milk much higher than average rate for breed with usual roughage feeding.

3. High physical condition of cows.

##### *Good feeding.*

1. T.D.N. supplied are from 200 pounds below to 1000 pounds above needs.

2. Rate of grain to milk is average for the breed with usual rates of roughage feeding and below average with heavy rates of roughage feeding.

3. Physical condition good or fair at both start and finish of year.

##### *Fair feeding.*

1. T.D.N. supplied are within 500 pounds of needs.

2. Rate of grain slightly below average for breed considering rate of roughage feeding.

3. Physical condition of cows lower at close of year than at the start.

*Poor feeding.*

1 T.D.N. supplied 1000 pounds or more below needs.

2. Rate of grain to milk very low for breed.

3. Physical condition much lower at close of year than at start or poor both at start and close.

4. Size of cows usually decidedly below average for breed.

The value of this feeding level analysis depends on the care which testers take in making feed reports in respect not only to the amounts of feed fed but to their quality. In instances where the analysis of a herd is of particular importance or where questionable results are obtained, an additional check on the feeding level is made from the testers' monthly reports on that herd by correlating the maintenance of daily milk production by months with the per cent of cows milking. The way in which cows hold up to normal milk production is a good indication as to whether or not a satisfactory level of feeding was maintained throughout the year.

**E12. Display of Extension Teaching Ideas.** E. C. SCHEIDENHELM, Michigan State College.

The extension section will again have an exhibit of extension teaching ideas.

*Missouri* will display three film strips. One will deal with quality of production, a second with methods of feeding, and a third with genetic information.

*South Dakota* will have a series of three charts which present facts to show how to decrease production costs through feeding methods.

*Nebraska* will portray the method used in making the junior bull ring project successful.

*Iowa* will display leaflets used in interesting people in keeping D.H.I.A. records.

*Michigan* will show their method of informing dairymen more fully about the proof on their herd sires. This will include letters to the dairymen, testers and county agricultural agents.

Other states indicating that they would exhibit but not reporting a subject were Wisconsin, Indiana (Purdue), Kansas, Texas, West Virginia, South Carolina, Alabama, and Tennessee.

**E13. Type Classification Committee Report.** JAS. W. LINN, Kansas State College.

The objects of the program are:

A. To offer a means of teaching individuality of animals.

- B. To assist breeders in analyzing their herds from a type standpoint.
- C. To be used as a means of furnishing additional information on proved bulls.

The program can be applied only to identified herds that are, at least, in their second year of dairy herd improvement association testing or to herds on breed association herd test.

The type rating shall be done by recognized official judges such as college professors, extension dairymen, or county agents with special training.

A. A state committee to approve judges for this work consisting of the head of dairy department, extension dairymen, and one active breeder to be selected by the above.

**E14. Clinics for Dairy Herd Improvement Association Fieldmen.** A. J. CRAMER, University of Wisconsin.

A total of 41 clinics or "check-up" schools for Dairy Herd Improvement Association fieldmen were held in 23 Wisconsin counties during 1939. These clinics for fieldmen were planned to give a review of the work required of the men in our dairymen's service program.

Where there are 3 or more associations within a county, it is our aim to hold at least two schools during the year. These 3 hour afternoon schools are held to give instructions to both beginners and experienced fieldmen. We use the more experienced fieldmen to serve as examples to the new men; they give their experiences of work with association members.

These meetings are held in the county agent's office, where field problems are discussed and threshed out. On the morning following the clinics, the supervisor inspects the work of the newer fieldmen on the farm while taking samples of milk, testing the milk, weighing the feed and ear tagging the new calves and cows. We also supervise the work done in the members' herd book, on feed sheets, barn feeding sheets, monthly report forms, identification and lactation sheets.

At some clinics the state supervisor invites the D.H.I.A. officers and directors to join the fieldmen and county agent. The significant thing about these clinics is that the farmers contribute a good many ideas to the group. The officers give methods of keeping farm account records and their ideas of the services they expect of the fieldmen. We help those fieldmen who are slipping and those who do not thoroughly understand their requirements.

The farmers and fieldmen gather around a large table so the discussion is informal. This brings about the exchange of ideas, and new practices are carried back to the farm by the fieldmen.

As a result of these fieldmen clinics, we supervisors save the state time and travel money by concentrating our effort where it is most needed. The clinics arranged with the cooperation of A. O. Follett, who supervises the

field work for Farm Account record keeping. We arranged to travel together in one car and saved the expense of running a second car.

**E15. 4-H Dairy Programs, Requirements and Recommendations.** H. A. WILLMAN, Cornell University.

A detailed 4-H program which will apply fully to all sections of one state or to the entire country, probably cannot be set up. The problems in connection with 4-H Dairy Club Work differs too widely between states and counties and often within boundaries.

A dairy program involving requirements and recommendations is, therefore, a large one. It composes several phases of work, each of which must be emphasized. The direction of too much attention to one single phase of work such as exhibiting will not lead to the greatest results and in the long run may lead to serious mistakes, disappointments and a lagging of interest. A program must be well balanced to bring about the type of improvement which boys and girls and our dairy business needs. Of necessity, programs should include a study of the surest methods of making progress in developing herds and all 4-H members should have an opportunity to take part in such activities or contests as Exhibiting, Records and Record Keeping, Judging, Showmanship and Demonstration team work.

Rather than to lay down too many rules and regulations, I believe we should in our 4-H dairy work take more boys and girls where they are and with the animals which they may have or usually can secure rather than to require that they start at a point which annually deprives many youths from 4-H club opportunities and benefits. Four-H dairy work should be made educational rather than regulatory in nature, therefore, I prefer to use the term recommend or urge rather than to make requirements which are often difficult to enforce. As a matter of fact, I believe that we might well under emphasize the word purebred if necessary in order to keep before the minds of 4-H club members, local leaders and parents, the importance of known ability in the selection and breeding of 4-H cattle.

For instance in our own state, we have not attempted to make any particular requirement regarding records and record keeping, but have attempted to make it a very important and definite part of the entire 4-H dairy program. As a matter of fact, we offer special incentives for this phase of work as well as for exhibiting and as a result we are getting an excellent response from the boys and girls. A short time ago a study was made of some of the 4-H records which were secured from the members in ten organized dairy clubs in which the following information was secured. These reports indicated that 221 members owned 598 head of cattle, 93 per cent of which were purebreds, 36 per cent of the cattle were in production, 91.6 per cent of those in production were on test, 66.5 per cent of those in production had completed one or more records averaging 354 pounds of fat

and 70 per cent of all 4-H cattle were selected from ancestry of known production. While this situation does not represent a cross section of the standard of dairy club work, it does suggest that better dairy work can be done. Four-H dairy work is built on the principle of herd improvement.

In the long run, a well rounded program will help us most not only in effectively attaining the general aims and purposes of 4-H club work but also in bringing about the type of improvement which the dairy business needs most.

**E17. An Extension Program in Quality.** J. M. Jensen, Michigan State College.

The Michigan State College Dairy Extension section has for its objective a quality program that involves the producer, manufacturer, and consumer in an approach to improvement of cream, butter, milk, and cheese.

Cream improvement is aimed at establishing grading practices and payment by grade. This is developed through educational meetings and demonstrations with farm groups, also through cream grading demonstrations with creameries.

Butter improvement is developed through a buttermaker's proficiency contest. The buttermaker is graded on his skill in scoring, analyzing and controlling fat and yeast and mold content. Technical meetings and scoring contests for creamery operators and buttermakers serve as a means of improving buttermaking operations.

The quality improvement program for milk is designed to assist the local sanitarians with promoting a better understanding by the producer of the need for quality milk, with interpretation of quality tests and with information in production problems. The microscope has been employed in making analysis of the milk supply in different areas with records kept of the analysis from one year to another. Assistance is given to producer distributors in designing and equipping milk pasteurizing plants.

Cheese improvement consists mainly in demonstration of quality tests on the milk supply, followed by the development in one county of a continuous quality improvement program that is conducted jointly by all the cheese factories in the county, with some financial assistance contributed by the county board of supervisors.

Consumption improvement consists partly in developing organized effort in the counties for furthering local usage of all dairy products. Demonstrations in food value and in evaluation of quality in dairy products before home economics groups, dairy and food councils and general consumer groups have been employed. F.F.A. judging of dairy products has been developed with the assistance of members of the dairy staff and the vocational education departments.

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## EFFECT OF HEAT AND pH ON THE INACTIVATION OF RENNIN IN WHEY

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### INTRODUCTION

As a prerequisite to the study of some of the properties of para-casein, a study of the conditions under which the rennin could be inactivated immediately after the para-casein was produced was begun, so that materials in which the rennin had been inactivated would be available for study. The desirability of inactivating the rennin is evident from the demonstrations that it is involved in the ripening of cheddar cheese, and particularly in view of the evidence presented by Barthel, Sandberg and Haglund (1) that the rennin used to coagulate milk in cheese making remains active in the cheese for at least 8 months.

The present paper reports the results of experiments on the inactivation of rennin in whey in which advantage was taken of the destructive effect of heat and pH. The results point to conditions under which rennin could be inactivated with a minimum of alteration of the constituents of the milk, particularly the proteins.

Lörcher (5) in his extensive study of the effect of salts, acids and alkalies on rennin, found that alkalies were more destructive than acids. Numerous other workers have obtained similar results. Michaelis and Rothstein (8) concluded that the rate of destruction of rennin (from pepsin) could be expressed by the following equation:

$$-\frac{dx}{dt} = k x^{3/2} (\text{OH})^4$$

Where  $x$  is the amount of rennin present at the time  $t$ ,  $\text{OH}$  is the hydroxyl ion concentration and  $k$  is a constant. Experimental verification of the equation was limited to the region from pH 6.3 to 7.3. They concluded that peptic activity was destroyed at the same rate as the rennin activity.

König (4) and others have demonstrated that the concentration and environment of the rennin exert a profound influence upon the rate of its inactivation by heat.

### EXPERIMENTAL

Milk was adjusted to pH 4.6 with normal HCl, the casein removed,

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the whey heated for some time in a steam heated oven and filtered. Various amounts of HCl or NaOH were added to 200 ml. amounts of the clarified whey, the whey was warmed to the desired temperature, and 1 ml. of Hansen's commercial rennet extract was added. At 2, 6, 10, and 14 minute intervals samples of the heated whey were removed, quickly cooled and the pH was adjusted to 6.0–6.5, using brom cresol purple as an indicator. One ml. was then added to 20 ml. of milk held at 40° C. and the time required for the formation of flocks was recorded. Reconstituted dry skim milk was used to determine the coagulating time. Previous to its use for testing 2.5 ml. of CaCl<sub>2</sub> solution (378 grams of CaCl<sub>2</sub> per liter) was added to each 100 ml. of milk. The CaCl<sub>2</sub> lowered the pH of the milk from 6.57 to 5.68. After the whey was heated for 14 minutes its pH was determined at 25° C. The results are presented in figure 1 and table 1.

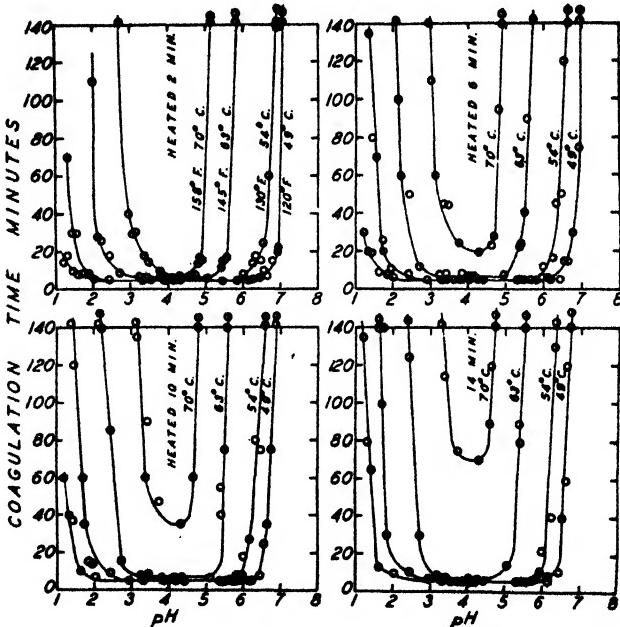


FIG. 1. Relation between temperature, pH and time of heating on the inactivation of commercial rennet extract heated in whey. The milk-coagulating power (time in minutes) of the whey after heating and cooling was used as the measure of inactivation.

The curves indicate a zone of maximum stability which centers at pH 4.0. The mid-line of the zone can be calculated from the data in table 1 by taking one-half the sum of each pair of values. The curves are nearly symmetrical with respect to an ordinate drawn through this pH. As the temperature of heating is increased and the time of heating is lengthened

TABLE I

*pH values, on the acid and alkaline side of the most stable zone, at which rennin activity was reduced 95 per cent by heating for the times and temperatures given. Below the acid and above the alkaline pH values given, inactivation of rennet is practically complete*

Time of heating in whey	Temperature at which rennin was heated in whey							
	49° C. (120° F.)		54° C. (130° F.)		63° C. (145° F.)		70° C. (158° F.)	
	acid	alk.	acid	alk.	acid	alk.	acid	alk.
minutes	pH	pH	pH	pH	pH	pH	pH	pH
2	0.8	7.1	1.1	6.9	2.0	5.9	2.8	5.1
6	0.9	7.0	1.3	6.7	2.1	5.8	3.0	4.9
10	1.0	6.9	1.4	6.6	2.2	5.6	3.2	4.8
14	1.2	6.8	1.6	6.4	2.4	5.5	3.3	4.7

the zone of maximum stability (minimum coagulation time) is narrowed. The change from minimum destruction to complete destruction which occurs on both sides of the zone of maximum stability takes place within a pH range of about one unit. The coagulation time may change from the minimum time of about 7 minutes to no coagulation after several hours by an alteration of about one pH unit. Rennin was heated at pH 4.3 in whey for 2 minutes at 70° C. with practically no inactivation, but was completely inactivated at pH 5.3 or above by the same heat treatment. At the lower temperatures a broad zone of pH was found in which no appreciable destruction occurred in the time intervals studied. When inactivation due to altering the pH begins, the effect is a function of a rather high power of the altering H or OH ion concentration.

Arbitrary equations were developed which are in general agreement with the experimental data. When they were extrapolated to 25° and 0° C. the predicted and the experimental results did not agree satisfactorily. Apparently temperature, time of holding and pH do not bear the simple relationship to one another at 25° C. or below that they do at 50° C. and above.

The zone of maximum stability for rennin is usually given as considerably higher than pH 4.0. This difference may be due to various causes such as difference in the composition of the liquid in which the rennin is held, the time and temperature of heating, or the source of the rennin. Holwerda (3) found the maximum stability in 10 per cent sodium chloride and 2 per cent boric acid to be from pH 5.3 to 6.3. Michaelis and Rothstein (8) conclude that rennin is stable below pH 6.0. Van Dam (11) found that pH values near the neutral point were destructive and that the composition of the solution was important. Michaelis and Mendelsohn (7) concluded that the optimum pH for rennin action was about 6.0 to 6.4. Lundsteen (6) found the optimum pH to be 5.4.

In conjunction with determinations of the loss in milk-coagulating power of rennet, tests for pepsin activity were made using the method of Gützner (2). Professor Sumner (9) kindly supplied the Carmine-Fibrin. The original rennet showed only slight pepsin activity as indicated by this test. After the coagulation power of the rennet was destroyed, no pepsin action was indicated during 24 hours holding with the Carmine-Fibrin when compared with a heated blank. These results are in agreement with the observations of Michaelis and Rothstein (8) that alkalies destroy both the coagulating and the peptic power of the rennet, and with the observation of Tauber and Kleiner (10) that rennin can be prepared which possesses little pepsin activity.

Barthel, Sandberg and Haglund (1) in their tests of the milk-coagulating power of extracts obtained from cheese after various periods of ripening found that extracts from Swiss cheese did not coagulate milk, whereas extracts from cheese of other varieties possessed milk-coagulating power. Sweet rennet curd is heated in the whey to 50–60° C. in making Swiss cheese. The curd of Gouda, Cheddar and other types of cheese is usually heated to lower temperatures, and the maximum temperature is attained only after considerable acidity has developed in the curd as a result of lactose fermentation by the lactic acid-producing bacteria with which the milk is inoculated. The data in figure 1 are in agreement with the findings of Barthel, Sandberg and Haglund and indicate the rather narrow limits between the destruction of rennin in Swiss and its survival in Gouda cheese. Their findings for destruction of rennin in the curd are in agreement with ours for destruction of rennin in whey.

#### SUMMARY

The zone of maximum stability of the milk-coagulating power of commercial rennet extract, when heated in whey to 49° C. or above, centers at pH 4.0. The pH-inactivation curves at the temperatures of 49° to 70° C. are nearly symmetrical both above and below pH 4.0. In the regions where alterations in pH accelerate inactivation, an alteration of one pH unit includes approximately the complete inactivation effect.

No peptic activity could be demonstrated, using the carmine-fibrin test, in solutions in which the rennin activity had been destroyed.

The data indicate that normal amounts of rennet can be inactivated by holding at 50° C. for 14 minutes at pH 6.8 to 7.0.

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## THE RELATIONSHIP BETWEEN THE MELTING TIME OF BUTTERFAT AND ITS MELTING POINT

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A rapid and convenient method for the determination of the melting time of butterfat was described in a previous paper from this laboratory (1). Essentially, the method is to determine the time required for a 25 ml. sample of butterfat at an initial temperature of 0° C. to melt and form a clear liquid when placed in a temperature-controlled water bath at 45° C. The test supplemented with hardness determinations (2) was designed for studying the "standing up" quality of butterfat at moderately high temperatures,—a quality which, despite its general commercial importance, has not been subjected to accurate measurement.

Several modifications of the proposed method appear possible, and would, no doubt, prove advantageous for routine work in which application of the test is made to fats and oils other than butterfat. For example, the temperature at which the melting time is to be determined might be raised or lowered in accordance with the melting point of the fat under investigation. Temperatures of 0° C. for chilling and 45° C. for melting have been found most suitable for butterfat. The melting time might be prolonged by substituting an air bath for the water bath. The latter change would be desirable in handling small samples of fat and an added convenience in observing changes of consistency during melting. Expression of results would necessarily need to be made in terms of a standard fat or oil having a constant melting time under the temperature conditions selected.

Chemical and physical constants, particularly the melting point, give some indication of the capacity of a fat to stand up at room temperatures. Occasionally, in the past, melting time has been studied by exposing standard blocks of butter or butterfat to a warm atmosphere and observing the condition of the samples at hourly intervals. This method of study, although capable of revealing marked differences between samples, especially when supplemented with photographic records, is time-consuming and does not allow for accurate measurement.

### EXPERIMENTAL

In order to study the relationship between the melting time of butterfat and its melting point, melting time determinations have been carried out on a large number of samples having high, medium, and low melting points.

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The possibility that melting time might be calculated with reasonable accuracy from melting point values, or vice versa, seemed likely.

The butterfat samples were handled in the usual manner, the butter being melted at 60° C., centrifuged and filtered, and kept in the dark in glass jars at 0° C. These samples were representative of the butterfat produced by individual cows of Ayrshire, Guernsey, Holstein, and Jersey breeds. The cows received experimental and control rations of varied composition. The melting point determinations were made according to the Wiley method as described in the A.O.A.C. methods (3). The melting time determinations were made according to procedures previously described (1).

#### RESULTS AND DISCUSSION

Table 1 gives the results obtained in which variations of 0.1° C. were allowed in melting point determinations and 15 seconds in the melting time determinations. These results are presented graphically in figure 1, in which average melting time is plotted against melting point.

The figures in table 1 show that there is considerable variation in the melting time of butterfat samples having the same melting point. In some instances this difference in melting time between samples amounts to over 3 minutes. This variation is greatest among samples having high melting points, 35°-37° C., and may be accounted for, in part, by the difficulties encountered in making accurate melting time determinations at a temperature only slightly above the melting point of the sample (1). The last column of table 1 gives the values obtained when the average melting time in minutes, is subtracted from the melting point, in degrees C. It is noteworthy that these values which fall between 20.2 and 21.5 vary only slightly, the average value being 21.1.

TABLE 1  
*Melting time of butterfat samples having melting points between 30.2° C. and 37.5° C.*

Number of samples	Melting point °C.	Melting time			Difference*
		Minimum min.	Maximum min.	Average min.	
3 .....	30.2	9.00	9.25	9.00	21.2
3 .....	31.0	9.00	10.25	9.50	21.5
3 .....	31.3	9.25	10.75	9.75	21.5
4 .....	31.7	9.50	10.75	10.25	21.5
7 .....	32.3	10.75	11.50	11.25	21.0
8 .....	32.5	10.50	12.00	11.25	21.2
5 .....	33.0	11.00	12.50	11.75	21.2
9 .....	33.5	11.00	13.25	12.25	21.2
6 .....	34.0	12.75	13.25	12.75	21.2
10 .....	34.5	12.75	14.50	13.50	21.0
19 .....	35.3	13.25	16.75	14.75	20.5
11 .....	35.6	14.00	16.75	15.00	20.6
8 .....	36.5	14.25	17.50	16.25	20.2
4 .....	37.5	15.50	18.75	17.25	20.2

\* Melting point in degrees C. minus average melting time in minutes.

It appears, therefore, that the melting time of butterfat may be estimated from melting point data,—in the present experiment, by subtracting 21.1 from the melting point. The melting time may be more closely approximated by use of the curve in figure 1, the points on the curve representing average values taken from table 1. Only two points, those at 32.3 and 32.5° C. lie outside the smoothed curve.

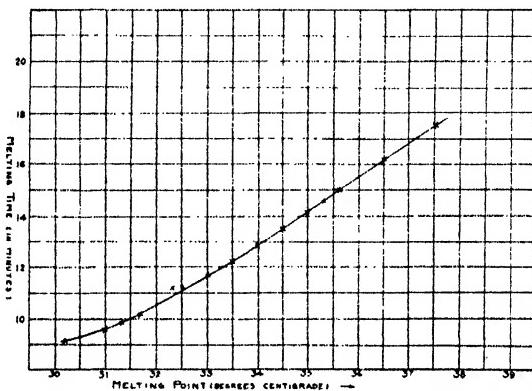


FIG. 1. Curve showing relation between melting point and melting time.

The foregoing discussion is made with full knowledge that the calculations are applicable only to melting time data secured under the experimental conditions as previously described (1). There is reason to believe that any change in these conditions which might effect a change in melting time would not alter the order of the results and that a direct relationship between melting point and melting time would still be evident.

#### SUMMARY AND CONCLUSIONS

Determinations were made of the melting time of butterfat samples having a wide range of melting point values.

Melting time was found to be roughly proportional to melting point.

Under the experimental conditions employed, the average melting time (expressed in minutes) of all samples was between 20.2 and 21.5 points lower than their actual melting points (expressed in degrees C.).

By means of a curve the melting time of butterfat samples can be closely approximated from melting point determinations.

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## OXIDIZED FLAVOR IN MILK: II. THE RELATION OF OXIDATION-REDUCTION POTENTIALS TO ITS DEVELOPMENT

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The development of oxidized flavor in milk is now assumed to be caused by certain oxidative changes taking place in the milk. Generally it has been believed that butterfat is the constituent in milk which has undergone oxidation on the development of the oxidized flavor. Findings from recent research tend to substantiate the belief that oxidized flavor in milk which has been catalyzed by copper results from oxidation of the phospholipids present in the milk. Since oxidized flavor results from oxidative changes, oxidation-reduction potentials should give valuable information in the study of this problem. Oxidation-reduction potential measurements have been applied by previous workers with various degrees of success. The work reported herein was conducted to further apply oxidation-reduction potential studies to the problem of oxidized flavor in milk and milk products.

### REVIEW OF LITERATURE

Thurston, Brown and Dustman (16) were the first to suggest that oxidized flavor in milk results from the oxidation of lecithin. Brown, Dustman and Thurston (1) found no appreciable differences in iodine numbers of butterfat from normal and oxidized milk. Swanson and Sommer (14) found a marked decrease in the iodine number of the phospholipid fraction of milk on the development of oxidized flavor but found no significant difference in the iodine numbers of butterfat from normal and oxidized milk. The above findings suggest that the phospholipid fraction of milk plays an important part in the development of oxidized flavor.

Gebhardt and Sommer (5) in 1930 found that there was a marked increase in the oxidation-reduction potential of milk when it was agitated with copper blades. Morris and Sommer (10) in 1932 obtained results which showed that the keeping quality of cream is poorest in samples having the highest oxidation-reduction potentials. These workers added a reducing agent, sodium sulphite, to the cream and prevented oxidation.

In 1933 Tracy, Ramsey and Ruehe (17) observed a definite relationship between the oxidation-reduction potential of milk and cream and the development of oxidized flavor on the addition of copper. The addition of copper caused a marked increase in the oxidation-reduction potential. Thurston (15) in 1935 reported on the influence of different metals and metallic salts on the oxidation-reduction potentials of milk. Stannous chloride, stannic chloride and aluminum chloride caused a decrease in the

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oxidation-reduction potentials of milk at a faster rate than in the control samples. Iron powder and ferric chloride caused a slight increase in potential. Ferrous chloride immediately after addition to milk caused an increase in potential, but this potential soon decreased to approximately the same potential as the control. Cuprous chloride caused the greatest increases in oxidation-reduction potentials.

Greenbank (7) found copper to be the most effective catalyst in producing oxidized flavor in milk. Ferrous iron also catalyzed the flavor development but larger quantities were required to produce the same results. Ferric iron was not very effective in producing the oxidized flavor. Greenbank suggests that ferrie iron, which is an oxidizing agent, is an inhibitor to this flavor development.

In 1937 Webb and Hileman (21) reported on their studies in which they used a vacuum tube potentiometric circuit for measuring oxidation-reduction potentials. These workers found that oxidized flavor in milk is due to or accompanied by an increase in oxidation-reduction potential, but this increase is not proportional to the concentration of added copper. Working with individual cows, they found that there is no relationship between oxidized flavors and oxidation-reduction potentials of milk.

Brown, Thurston and Dustman (2) added copper to raw milk, to milk before pasteurization and to milk after pasteurization. They found that raw milk and milk with copper added after pasteurization developed a more intense oxidized flavor, the suggested explanation being that some of the copper may combine with the proteins during the pasteurization process and is no longer free to catalyze the reaction.

Sharp, Trout and Guthrie (13) found that contamination of milk with copper caused destruction of vitamin C, and that there is a positive correlation between the rate of oxidation of ascorbic acid and the development of oxidized flavor. They also state that the holder method of pasteurization at 143-145° F. for 30 minutes does not appreciably accelerate the destruction of ascorbic acid.

Turgeon, Stebnitz and Sommer (19) worked on the Ritter's test as a means of determining copper contamination. Both the vitamin C and the copper content of the milk affected the results of the Ritter's test. They found that the vitamin C content was quite uniformly reduced to 7 milligrams per liter at the time of first color change and reduced to zero by the time the color was definitely developed.

Chilson (3), Dahle and Palmer (4) and Greenbank (7) have all reported that the addition of 50 to 100 milligrams of pure crystalline ascorbic acid to milks which develop oxidized flavor spontaneously will prevent the flavor defect.

In Central Europe electrical treatment of milk has been used as a means of neutralizing developed acidity in milk and cream. Winkler (22)

discusses an electro-neutralization process in which milk is subjected to the action of direct current. Gratz (6) also has discussed the process. Electrodes are placed in a vessel of non-conducting material. Milk enters the vessel at the bottom, flows between the electrodes, which are placed either in a vertical or horizontal position, and leaves through an overflow at the top. The process is covered by Austrian and Italian patents. Pien and Baisse (11) discuss the theory of electrical deacidification. Sodium liberated at the cathode neutralized the lactic acid, and the chlorine which was liberated at the anode combined with the protein. Woljagin and Scheimpflug (23) carried on electrical deacidification by means of electro-dialysis. Milk was placed in the cathode chamber and whey in the anode chamber with a clay diaphragm separating the liquids.

#### EXPERIMENTAL PROCEDURES

Since milk is a poorly poised system, a vacuum tube potentiometer circuit was made and used in conjunction with a Leeds-Northrup portable potentiometer. The vacuum tube potentiometer circuit was made according to plans furnished by Johnson (8). Platinum electrodes similar to those of Webb and Hileman (21) were used.

Difficulty was experienced in getting the electrodes in a sample of milk to check. Previous workers have also experienced the same difficulty. Closer checks between electrodes were obtained when extreme care was taken in their cleaning. The electrodes were first placed in boiling trisodium phosphate solution and then in hot chromic acid solution, remaining in each solution for a period of from one-half to one hour. The electrodes, after being removed from the chromic acid solution, were thoroughly rinsed and placed in distilled water from eight to twelve hours before using.

Two hundred-and-fifty-cc. brown glass bottles were used as electrode vessels. Each bottle was fitted with a cork stopper through which three holes were drilled. Two electrodes and a saturated potassium chloride agar bridge were placed into each sample of milk. A series of eight bottles were then connected to a saturated calomel half cell.

Milks from individual cows were obtained from the University herd. Aluminum pails were used for milking and the milk was brought to the laboratory in either aluminum pails or brown glass bottles. Extreme care was taken to prevent copper or iron contamination. The milk was taken to the laboratory and pasteurized within two hours after milking. Pasteurization was conducted in aluminum beakers with aluminum stirring devices. Heating was carried out in water baths which were connected to a steam line for heating and thermo-electrically controlled for holding at pasteurization temperatures. Immediately after pasteurization the containers were moved to a cold water bath for cooling.

The milk samples were then set up by adding the catalyst, and in some experiments anti-oxidants were also added. The samples were then placed in a refrigerator and allowed to stand with the least amount of handling possible. Duplicate samples were put in 500-cc. brown glass bottles and placed in the refrigerator along with the electrode vessels. These samples were used for vitamin C titrations and then observed at the end of the storage period of 60 to 72 hours for the development of oxidized flavor.

Oxidation-reduction potential readings were taken at short intervals during the early stages of the storage period and then taken at longer intervals after twenty-four hours of storage. The oxidation-reduction potential readings were then converted to Eh. The values for the E.M.F. of a calomel half cell at different temperatures were obtained from data by Vellinger (20).

Trout and Sharp (18) report that the temperature of 21° C. appeared to be more satisfactory for judging milk for oxidized flavor than did the temperature of 35° C. After storage the samples were warmed up to 21° C. and were always examined by at least two judges who were familiar with oxidized flavor. The following system was used for recording the presence of oxidized flavor and its intensity:

- + + + + = very strong oxidized flavor.
- + + + = strong oxidized flavor.
- + + = distinct to pronounced oxidized flavor.
- + = slight oxidized flavor.
- ± = doubtful oxidized flavor.
- = no oxidized flavor.

The method of Kon and Watson (9) was used in this work for determining the reduced ascorbic acid content of milk. The milk proteins and fat were precipitated with 20 per cent tri-chloroacetic acid. An exact quantity of filtrate was then titrated with 2:6 dichlorobenzenoneindophenol. The dye was standardized against crystalline ascorbic acid. In this work the water used was re-distilled in an all-glass distilling apparatus.

#### *Influence of metal on oxidation-reduction potentials and the development of oxidized flavor*

Pasteurized milk from an individual cow was used in preparing the samples for this experiment. A copper sulfate solution was prepared so that one cc. of solution added to one pint of milk would give a copper concentration of 0.5 parts per million parts of milk. Ferric sulfate and ferrous sulfate solutions were made up to such concentrations that one cc. of solution added to one pint of milk would give 5 parts of iron per million parts of milk.

Copper was added at concentrations of 0.0, 0.25, 0.5, 1, 2 and 5 parts per million parts of pasteurized milk. Ferric iron and ferrous iron were added

at concentrations of 0, 5, 10, 25 and 50 p.p.m. The changes in O-R (oxidation-reduction) potentials were followed, and at the end of 72 hours the samples were examined for oxidized flavor.

A similar experiment was conducted on a sample of mixed herd milk. The results of the two experiments were compared to determine the difference in susceptibility of the two milks to oxidized flavor development.

#### *Time of copper addition to milk*

Copper in the form of copper sulfate solution was added to milk before and after pasteurization at a concentration of 1 p.p.m. Two series of samples were prepared and kept in a water bath at 10° C. for 15.5 hours. Electrodes were placed in one series, and from the other series, samples were taken for reduced ascorbic acid determinations. O-R potential readings and ascorbic acid titrations were made at short intervals.

#### *Copper addition to whole milk, skimmilk and cream*

A mixed sample of milk was pasteurized in an eight gallon aluminum milk can and then separated. Samples of whole milk, skimmilk, 20 per cent cream and 29 per cent cream were prepared with and without added copper. Two parts of copper per million were added. Potential measurements were made during the storage period, and at the end of 72 hours all samples were examined for oxidized flavor.

#### *A study of milk from individual cows*

Milks were obtained from four individual cows. Two of these cows were Holsteins and the other two were Guernseys. Each lot of milk was pasteurized separately and then divided into two parts. To one part, copper was added at a concentration of 2 p.p.m. and to the other part no metallic catalyst was added. Samples were then prepared for O-R potential measurements and for ascorbic acid determinations. The samples were stored at 4° C. for 72 hours and at the end of this period they were examined for the presence of oxidized flavor. During the storage period O-R potential measurements and reduced ascorbic acid determinations were made at regular intervals.

#### *Ascorbic acid as an anti-oxidant*

Crystalline ascorbic acid and crystalline d-isoascorbic acid were added to pasteurized milk to study the effect on O-R potentials and the development of oxidized flavor. Pasteurized milk was divided into three lots. To one lot 100 mgs. of ascorbic acid per liter of milk was added, to another 100 mgs. of d-isoascorbic acid per liter of milk, and the other lot was left as the control. Then each lot of milk was divided in half and to the one half 3 p.p.m. of copper as copper sulfate was added. Oxidation-reduction po-

tential measurements and titrations for reduced ascorbic acid were made at regular intervals during storage. At the end of 60 hours the samples were examined for the presence of oxidized flavor.

#### *Change in oxidation-reduction potentials caused by electrical current*

Electricity was passed through milk as a means of changing the O-R potential. A glass chamber was constructed, using a 1.5" by 8" glass test tube with a milk capacity of 95 cc. Milk was allowed to flow into the chamber from below and carried out by means of a side-arm connection. Two platinum electrodes, 0.5" by 2.0", were placed within the chamber. A porous clay thimble was also placed in such a position that it would extend down into the milk. Inside of the thimble was placed a dilute solution of sodium chloride and a carbon electrode. The sodium chloride solution was continuously replaced by fresh solution. Dry cells were used as the source of the electrical current. For the work reported herein, a current of 24 volts and 0.4 amperes was used. Milk was passed through the chamber at the rate of 155 cc. per minute.

In the first series of the experiment, milk was passed through the apparatus without any current flowing. In the second series, the platinum electrodes were negative and the carbon electrode in the salt solution was positive. In the third series, the platinum electrodes were positive with the carbon electrode negative. In the last series the clay porous thimble was removed and one of the platinum electrodes was positive and the other negative. Two samples of mixed milk were set up from each series. Copper in the form of copper sulfate solution was added to one of the series at concentrations of 2 p.p.m. Potential measurements were made and after 72 hours the samples were examined for oxidized flavor.

#### EXPERIMENTAL RESULTS

All of the oxidation-reduction potential measurements were made with two electrodes in each sample of the milk. The potential readings from the two electrodes were averaged, and this average was taken as the potential for each reading time. The results from each series of experiments were tabulated and from these data the following graphs were drawn.

#### *Influence of metal on oxidation-reduction potentials and the development of oxidized flavor*

Figure 1 gives the results of the different concentrations of copper in the form of copper sulfate on the O-R potentials of milk from an individual cow. Copper caused a considerable increase in the O-R potential. The increase in potential was slower and not as great with the lower concentrations of copper. The rise in potential was not in proportion to the concentration of added copper. When the potential had reached its maxi-

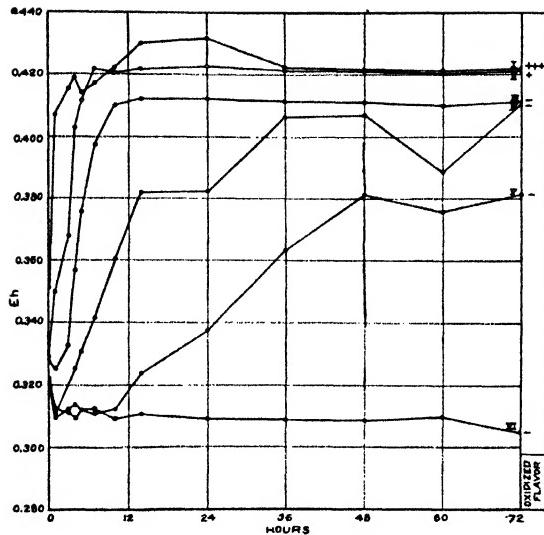


FIG. 1. The effect of different concentrations of added copper on oxidation-reduction potentials of milk and the development of oxidized flavor.

- I—5 parts per million of added copper.
- II—2 parts per million of added copper.
- III—1 part per million of added copper.
- IV—0.5 part per million of added copper.
- V—0.25 part per million of added copper.
- VI—0 part per million of added copper.

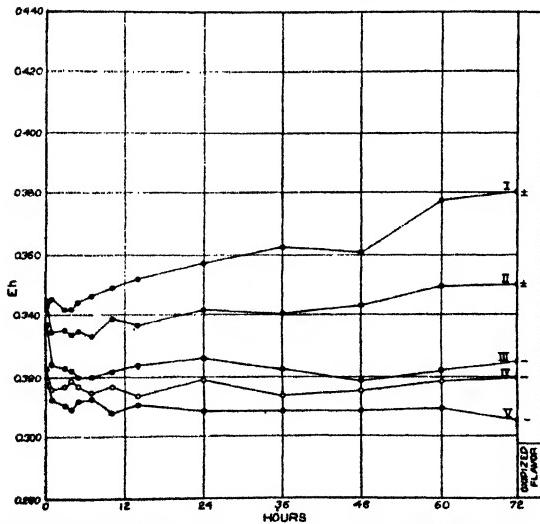


FIG. 2. The effect of different concentrations of added ferric iron on the oxidation-reduction potentials of milk and the development of the oxidized flavor.

- I—50 parts per million of added ferric iron.
- II—25 parts per million of added ferric iron.
- III—10 parts per million of added ferric iron.
- IV—5 parts per million of added ferric iron.
- V—0 parts per million of added ferric iron.

mum point, it continued to remain fairly constant at this high level. With the lower concentration of added copper and with the control, there was a marked decrease in potential during the first hour.

The addition of ferric iron in the form of ferric sulfate, as shown in figure 2, does not cause a rapid increase in potential like the addition of copper. There was a small gradual increase in potential during the entire storage period. The increase was greater with the higher concentrations of ferric iron. Only in the concentrations of 25 and 50 parts per million of ferric iron was there a slight resemblance of oxidized flavor. Ferric iron added at the rate of 50 parts per million did not affect the potentials any more than did 0.25 parts per million of copper.

Figure 3 shows that the addition of ferrous iron to milk caused a decrease in potential. These results are in keeping with the property of ferrous sulfate because it is a reducing substance.

Table 1 summarizes the effectiveness of copper, ferric iron and ferrous iron in catalyzing the development of oxidized flavor in mixed milk and milk from an individual cow. The mixed milk was more susceptible to the metallic catalyst than was the milk from the individual cow. In both cases ferric iron caused little oxidized flavor while the same concentrations of ferrous iron were very effective.

#### *Time of copper addition to milk*

Copper added at concentrations of one part per million to a sample of milk from an individual cow before pasteurization had catalyzed the oxida-

TABLE 1

*The effectiveness of different metals in catalyzing the oxidized flavor in a sample of mixed milk and a sample of milk from an individual cow*

Catalyst	Concentration of catalyst	Source of milk	
		Individual cow	Receiving tank
Copper in form of copper sulfate	p.p.m.		
	0.0	-	-
	0.25	-	+
	0.5	-	+
	1.0	-	++
	2.0	+	++++
Ferric iron in form of ferric sulfate	5.0	+++	+++++
	0.0	-	-
	5.0	-	-
	10.0	-	-
	25.0	+	+
	50.0	±	±
Ferrous iron in form of ferrous sulfate	0.0	-	-
	5.0	±	++
	10.0	+	++
	25.0	++	+++
	50.0	+++	++++

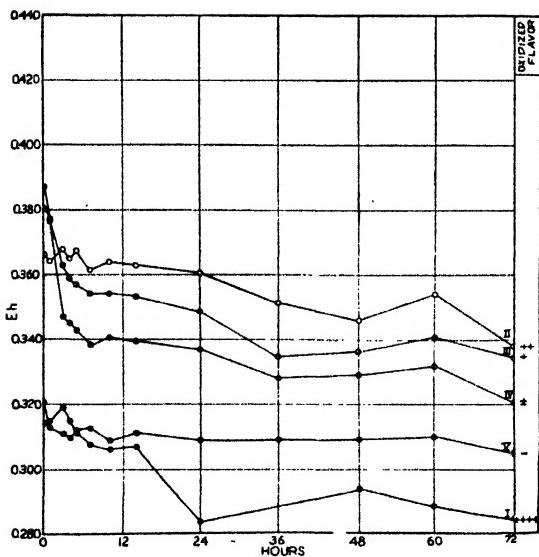


FIG. 3. The effect of different concentrations of added ferrous iron on the oxidation-reduction potentials of milk and the development of oxidized flavor.

I—50 parts per million of added ferrous iron.

II—25 parts per million of added ferrous iron.

III—10 parts per million of added ferrous iron.

IV—5 parts per million of added ferrous iron.

V—0 parts per million of added ferrous iron.

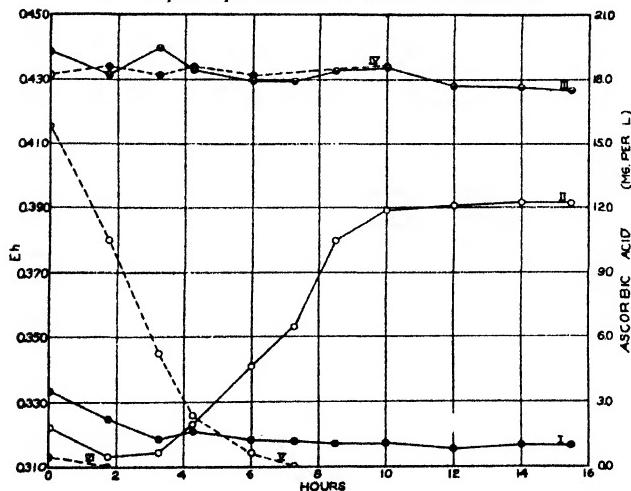


FIG. 4. The influence of time of copper contamination on the oxidation-reduction potentials of milk and the oxidation of the reduced ascorbic acid present in the milk.

I—Without added copper.

II—Copper (2 p.p.m.) added after pasteurization.

III—Copper (2 p.p.m.) added before pasteurization.

IV—Ascorbic acid content of I.

V—Ascorbic acid content of II.

VI—Ascorbic acid content of III.

tion of nearly all of the reduced ascorbic acid before the pasteurization process had been completed. In the sample of milk to which copper was added after pasteurization the reduced ascorbic acid was completely oxidized after 7 hours. During the same period of time there was no marked change in reduced ascorbic acid content of the milk to which no copper had been added. Figure 4 shows that the oxidation-reduction potential of pasteurized milk containing copper did not increase until nearly all of the reduced ascorbic acid had been oxidized. The O-R potential of the sample of milk to which copper had been added before pasteurization, had reached its maximum before the potential readings were started and continued to remain higher than the potential of the milk to which copper had been added after pasteurization.

#### *Copper addition to whole milk, skimmilk and cream*

Figure 5 summarizes the results of this experiment. Skimmilk and whole milk were found to have O-R potentials of about the same Eh values. The addition of two parts per million of copper caused practically identical increases in potential. The 29 per cent cream showed higher Eh values in the sample to which no copper had been added than did the whole milk and skimmilk. This was also true of the series in which copper was added. The skimmilk and whole milk developed the same intensity of oxidized flavor on the addition of copper, while the oxidized flavor was more intense in the case of the cream.

#### *A study of milk from individual cows*

The effect of adding copper to milk from individual cows is shown in figure 6, and it also shows the difference in potential of the normal milk

TABLE 2

*A comparison of the effect of the addition of copper on the oxidation of the reduced ascorbic acid in the pasteurized milks of four individual cows*

Time after adding copper	Ascorbic acid per liter							
	Cow #33		Cow #68		Cow #416		Cow #426	
	Cu 0 p.p.m.	Cu 2 p.p.m.	Cu 0 p.p.m.	Cu 2 p.p.m.	Cu 0 p.p.m.	Cu 2 p.p.m.	Cu 0 p.p.m.	Cu 2 p.p.m.
hrs.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
0.25	17.80	12.48	17.45	13.40	15.30	12.45	23.00	15.30
3	17.80	6.55	17.45	7.15	13.70	3.45	21.15	9.35
5	17.45	0.94	17.45	2.50	13.70	1.56	20.15	3.22
14.5	17.45	0	17.45	0	11.85	0	17.75	0
20								
26	16.20	0	14.65	0	10.00	0	16.20	0
37	16.20	0	13.10	0	8.42	0	14.35	0
45								
52	12.80	0	10.00	0	6.25	0	13.10	0
64	8.75	0	7.50	0			10.60	0
72	7.50	0	7.20	0	2.60	0	10.60	0

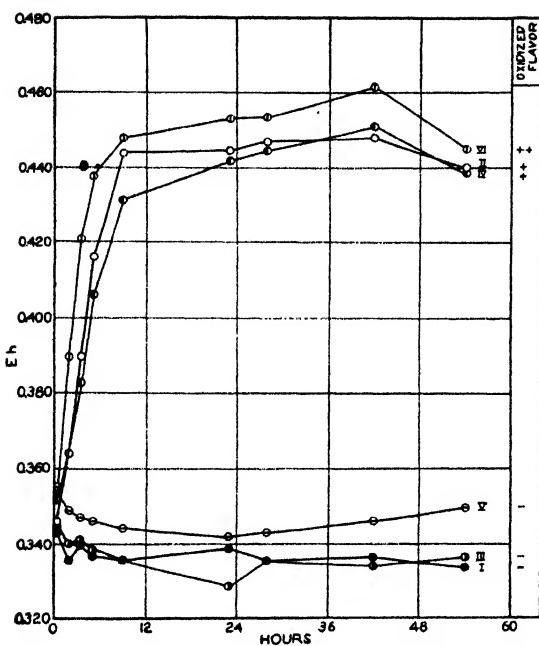


FIG. 5. A study of the influence of added copper on the oxidation-reduction potentials of whole milk, skimmilk and cream.

- I—Whole milk, no added copper.
- II—Whole milk with added copper (2 p.p.m.).
- III—Skimmilk, no added copper.
- IV—Skimmilk with added copper (2 p.p.m.).
- V—29 per cent cream, no added copper.
- VI—29 per cent cream with added copper (2 p.p.m.).

from individual cows. Table 2 gives the results of the reduced ascorbic acid determination on the same milks. Comparing the results in figure 6 with the results in table 2, the milk from cow #426 had the highest ascorbic acid content and the lowest oxidation-reduction potential in the normal milk. The addition of copper to this milk caused the smallest increase in potential and it did not develop oxidized flavor. The milk from cow #416 had the lowest reduced ascorbic acid content and highest oxidation-reduction potential in the normal milk. The milks from cows #33 and #68 had about the same ascorbic acid content and oxidation-reduction potential. The addition of copper caused about the same increase in potential and the same degree of oxidized flavor. The addition of copper to the milk from cow #416 caused the potential to raise to the same level as in the milks with added copper from cows #33 and #68, but the degree of oxidized flavor was less. Cows #33 and #68 were Holsteins and cows #416 and #426 were Guernseys.

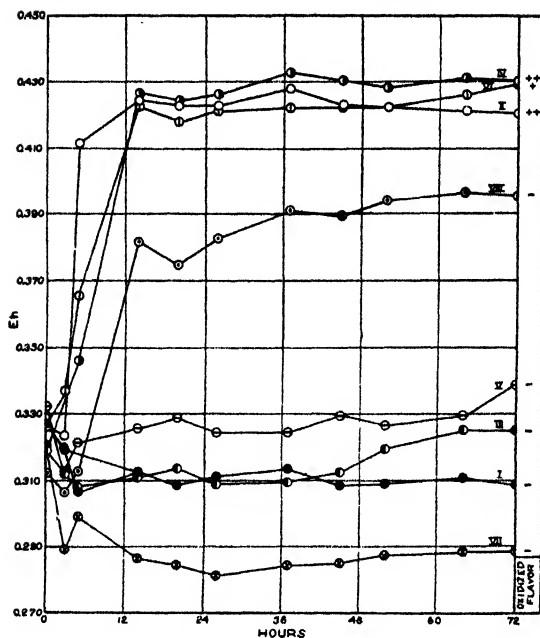


FIG. 6. The change in oxidation-reduction potentials and the development of oxidized flavor following the addition of copper to the pasteurized milks of four cows.

- I—Milk from cow #33, no added copper.
- II—Milk from cow #33 with added copper (2 p.p.m.).
- III—Milk from cow #68, no added copper.
- IV—Milk from cow #68 with added copper (2 p.p.m.).
- V—Milk from cow #416, no added copper.
- VI—Milk from cow #416 with added copper (2 p.p.m.).
- VII—Milk from cow #426, no added copper.
- VIII—Milk from cow #426 with added copper (2 p.p.m.).

#### *Ascorbic acid as an anti-oxidant*

The experimental results obtained from the study of ascorbic acid and d-isoascorbic acid as anti-oxidants are given in figure 7 and table 3. The addition of both forms of ascorbic acid caused a marked decrease in the oxidation-reduction potentials of the milks to which they were added. During the entire storage period the added ascorbic acids kept the potential down in the samples to which copper had been added, but the reduced ascorbic acid was being continually oxidized in these samples. At the end of the 54 hours of storage nearly all of the reduced ascorbic acid had been oxidized. More of the d-isoascorbic acid had been oxidized than had the ascorbic acid. During the period of the oxidation of the ascorbic acids, the potentials were lower in the samples containing copper than in the other samples. Although the milk containing d-isoascorbic acid and added cop-

per had the lowest Eh values during the entire storage period, it developed an oxidized flavor that was as intense as the flavor in the control sample to which copper had been added.

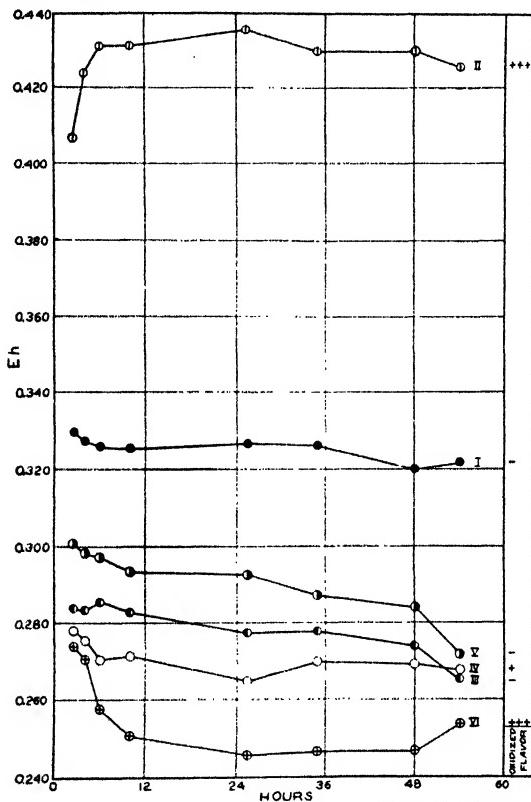


FIG. 7. The influence of added copper and ascorbic and d-isoascorbic acid on the oxidation-reduction potentials of milk and the development of oxidized flavor.

I—Without added ascorbic acid or copper.

II—Without added ascorbic acid, but with added copper (3 p.p.m.).

III—With added ascorbic acid (100 mg. per liter), but without added copper.

IV—With added ascorbic acid (100 mg. per liter) and copper (3 p.p.m.).

V—With added d-isoascorbic acid (100 mg. per liter) but without added copper.

VI—With added d-isoascorbic acid (100 mg. per liter) and copper (3 p.p.m.).

#### *Change in oxidation-reduction potential caused by electrical current*

By passing electrical current through milk it was possible to alter the oxidation-reduction potential. The greatest decrease in potential was effected by placing the negative electrode in the milk and the positive electrode in the sodium chloride solution contained in the porous cup. The curves plotted from the data obtained in this experiment are shown in

TABLE 3

*The influence of added copper on the ascorbic acid content of normal milk and milk to which ascorbic acid and d-isoascorbic acid have been added*

Time after adding copper	Ascorbic acid content					
	Without added ascorbic acid		With added ascorbic acid		With added d-isoascorbic acid	
	Without added copper	3 p.p.m. added copper	Without added copper	3 p.p.m. added copper	Without added copper	3 p.p.m. added copper
hrs.	mg.	mg.	mg.	mg.	mg.	mg.
0.5	17.60	10.25	104.00	95.25	104.00	87.50
2.5	17.60	0	104.00	57.25	104.00	51.75
4						
6	17.60	0	104.00	51.60	103.50	43.00
10						
25.5	16.65	0	103.50	41.00	102.00	38.10
34.5	15.80	0	103.50	18.65	102.00	16.60
48						
54	15.80	0	96.25	12.95	96.00	7.90

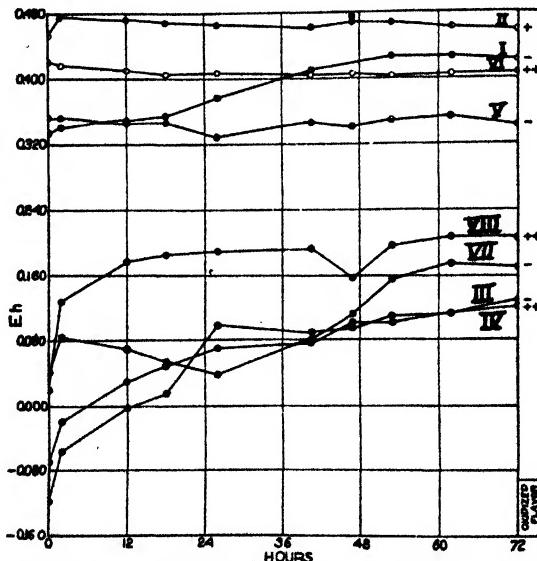


FIG. 8. The use of electrical currents as means of changing the oxidation-reduction potentials of milk and the influence on oxidized flavor development.

I—No electrical treatment, no added copper.

II—No electrical treatment, with added copper (2 p.p.m.).

III—Negative electrode in milk, no added copper.

IV—Negative electrode in milk, with added copper (2 p.p.m.).

V—Positive electrode in milk, no added copper.

VI—Positive electrode in milk, with added copper (2 p.p.m.).

VII—Both electrodes in milk, no added copper.

VIII—Both electrodes in milk, with added copper (2 p.p.m.).

figure 8. Though it was possible to decrease the potential, in no case did the treatment prevent the development of oxidized flavor when copper was added to the samples.

#### DISCUSSION

A number of investigators, Gebhardt and Sommer (5), Tracy, Ramsey and Ruehe (17), Thurston (15) and others, have shown that the addition of copper to milk causes an increase in the oxidation-reduction potential. Copper is known to be a very effective catalyst in causing the development of oxidized flavor. From these observations it is logical to assume that the development of oxidized flavor is due to or accompanied by an increase in the oxidation-reduction potential of the milk.

Copper was found to cause a sharp increase in the oxidation-reduction potential shortly after it was added to the milk. After the potential has reached its new level it continues to remain nearly constant during the remainder of the storage period. The length of time required for this sharp increase is dependent upon the concentration of added copper. In these experiments the addition of 2 and 5 p.p.m. of copper caused an immediate increase in potential, while with the lower concentrations there was a lag period.

It has been shown in figure 4 that the oxidation-reduction potential does not increase until the reduced ascorbic acid in the milk has nearly all been oxidized. These results are in keeping with the observations made by Turgeon, Stebnitz and Sommer on the Ritter's test as a means of determining copper contamination. These workers found that the vitamin C content of the milk was quite uniformly reduced to 7 milligrams per liter at the time of the first color change and reduced to zero by the time color was definitely developed. The conclusion may be drawn that the development of color in the Ritter's test is due to an increase in the O-R potential of the medium.

The addition of ferric iron to milk caused a gradual increase in the potential during the entire storage. The higher the concentration of added ferric iron, the greater was the resulting potential. The addition of 50 p.p.m. of ferric iron did not cause the potential to rise higher than the addition of 0.25 p.p.m. of copper. Only in the samples of milk where ferric iron had been added at concentrations of 25 and 50 p.p.m. was there any evidence of an oxidized flavor.

Ferrous iron caused a decrease in the oxidation-reduction potential when it was added to milk. Immediately upon its addition to milk the resulting potential was higher (except on the addition of 50 p.p.m.) than in the samples to which copper and ferric iron had been added, but on storage the oxidation-reduction potential kept decreasing. The sample of milk containing 50 p.p.m. of added ferrous iron developed a more intense oxidized flavor than did the sample of milk containing 5 p.p.m. of copper; yet there

was a difference of 0.135 in Eh. Concentrations of 25 and 50 p.p.m. of added ferrous iron to milk caused intense oxidized flavor, while the same concentrations of ferric iron caused only a questionable development of the off-flavor. The results were found to be the same with the use of pasteurized milk from an individual cow or with pasteurized mixed herd milk. In the case of the mixed herd milk the development of the oxidized flavor was more intense. From these findings it would seem that the development of oxidized flavor in milk is dependent upon the catalyzing properties of certain metallic ions and not on the change in potential which they may create.

The addition of copper to skimmilk and whole milk caused about an identical change in O-R potential. The cream had an original O-R potential higher than did the skimmilk and whole milk, and the resulting potential on the addition of copper was also higher. Since skimmilk had developed the same intensity of oxidized flavor as did the whole milk, this gives evidence to the fact that the phospholipids are the substances which undergo chemical change on the development of oxidized flavor. These findings are in agreement with the work of Thurston, Brown and Dustman (16) and Swanson and Sommer (14). The cream developed a more intense oxidized flavor than the whole milk, which is in agreement with the findings of Roland and Trebler (12).

The normal pasteurized milks from individual cows vary in their oxidation-reduction potentials. In comparing the milk from four individual cows, there was a relation between the reduced ascorbic acid content of the milk and its oxidation-reduction potential. Milk from the cow having the lowest ascorbic acid content had the highest O-R potential of the four milks. The milk having the highest reduced ascorbic acid content had the lowest O-R potential, and the milks from the other two cows had about the same reduced ascorbic acid content and O-R potential. There was no correlation found between breed and reduced ascorbic acid content as the high and low milks were from Guernseys and the other two from Holsteins. The milk with the highest reduced ascorbic acid content showed less increase in O-R potential on addition of copper and did not develop an oxidized flavor.

The addition of crystalline ascorbic acid and crystalline d-isoascorbic acid to milk was found to cause a marked decrease in the oxidation-reduction potential. The addition of copper to milks containing these two forms of ascorbic acid caused oxidation of the ascorbic acid but during the entire period the O-R potential was lower than in the samples which contained added ascorbic acid but no added copper. As the oxidation of the ascorbic acid is taking place, the potential is lower than where there is no rapid oxidation of the reduced ascorbic acid. The addition of 100 mg. per liter crystalline ascorbic acid to milk inhibited but did not completely prevent the development of oxidized flavor. Though d-isoascorbic acid produced

the lowest O-R potential readings, it did not prevent the development of oxidized flavor.

Passing electricity through milk has been used in Central Europe as a means of neutralizing developed acidity in milk and cream. This same principle was used as a means of changing the O-R potential in milk. By means of passing electricity through milk it was possible to lower the O-R potential of milk considerably, but such changes in potential were found to exhibit no inhibiting effects on the development of oxidized flavor.

#### SUMMARY AND CONCLUSIONS

The relation of oxidation-reduction potential measurements to the development of oxidized flavor in milk was studied to determine if there was any relation between an increase in O-R potential and the development of oxidized flavor in milk.

Copper was capable of producing approximately the same intensity of oxidized flavor when added at one-tenth of the concentration at which ferrous iron was added to milk. Shortly after the addition of copper to milk there was a rapid increase in oxidation-reduction potential, while ferrous iron caused the oxidation-reduction potential to decrease. Ferric iron when added to milk caused the O-R potential to increase slowly during the storage period but produced little or no oxidized flavor.

When copper was added to milk, the oxidation-reduction potential did not increase until practically all of the reduced ascorbic acid was oxidized.

The addition of copper to whole milk, skimmilk and cream was followed by a marked increase in O-R potential. The cream developed a more intense oxidized flavor than the whole milk and skimmilk.

The oxidation-reduction potential of milk from individual cows was found to vary; as the reduced ascorbic acid content of milk from individual cows increased, the O-R potential decreased.

Crystalline ascorbic acid and crystalline d-isoascorbic acid when added to milk lowered the oxidation-reduction potential. Ascorbic acid had an inhibiting effect on the development of oxidized flavor, but even at the low Eh reading produced by the addition of d-isoascorbic acid, the milk developed oxidized flavor when copper was added.

By passing electrical current through milk, it was possible to lower the oxidation-reduction potential, but the resulting lowered O-R potential did not inhibit the development of oxidized flavor.

From these studies on oxidation-reduction potentials in relation to the development of oxidized flavor, the Eh value of the medium does not seem to inhibit or accelerate the development of the off-flavor.

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## A COMPARATIVE EVALUATION OF AN ICE CREAM SUPPLY AS IT REACHES THE CONSUMER\*

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The prospective ice cream consumer is often confronted with a multiplicity of prices for the usual consumer-size package of ice cream. The problem is no less complex after the seller has been questioned as to the reasons for the price differences.

In an effort to gain some direct information bearing on this problem, vanilla ice cream was purchased in pint quantities in a typical consumer manner, that is, without indication to the dispenser as to the use that was to be made of the ice cream. These samples were brought immediately to the laboratory and the gross weight obtained. Portions were withdrawn with a sterile spatula for bacterial analysis and at the same time an organoleptic examination was made. The remainder of the contents of the package was transferred to a screw-top pint jar and the container was weighed to obtain the net weight of the ice cream. The ice cream was melted at room temperature and, when necessary in removing incorporated air, warmed to 90 degrees Fahrenheit. The overrun was calculated by the use of the specific gravity as determined with a 100-milliliter pycnometer bottle and a torsion balance. Total solids and fat were determined by the Mojonnier procedure and protein by the Gunning method using a 5-gram sample. The pH was determined electrometrically with a quinhydrone electrode. The total bacterial count was made by the standard plate method on a volumetric basis. The number of organisms of the coli group was obtained by the plate method using violet red bile agar, and in the case of low count samples this was supplemented by the brilliant green lactose bile broth method. The samples are designated in table 1 according to number with the suffix P or B indicating factory-filled packages or fountain-dipped bulk ice cream, respectively.

### RESULTS AND DISCUSSION

Inspection of the data in table 1 will show that the price of pint samples of vanilla ice cream as available to the consumer in the market studied, ranged from fifteen to thirty cents with intermediate prices of seventeen, twenty, and twenty-five cents. The net weight of these pint samples averaged 298.0 grams with a range of 212 grams to 465 grams. The heaviest sample was a fountain-dipped sample in the highest price level, while the lowest weight sample was a factory-filled pint in the lowest price level. This weight-price

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TABLE I  
*Composition and characteristics of pint samples of vanilla ice cream as purchased by the consumer*

Plant	Sample	Pint price	Net wt.	Over-run cale.	Total solids	Butter fat	Protein N×6.38	pH	Bacteria per ml.		Flavor		Body	
									%	%	thous.	thous.	Score	Criti- cism*
A	1-P	\$	.20	262	96.4	39.45	14.25	4.56	6.40	5.2	-10	44.0	.....	23.0
A	6-P	.15	223	129.3	38.42	12.97	3.97	6.37	26.9	120	41.0	.....	22.0	
A	8-B	.20	410	24.7	38.37	12.30	4.77	6.40	126.0	3450	43.0	L.F.P.	23.0	
B	2-B	.25	401	28.2	39.24	14.18	4.13	6.35	19.0	-10	42.0	O.I.	23.5	
B	3-P	.15	245	106.5	39.36	13.45	4.49	6.32	3.6	200	43.0	L.F.F.	23.0	
B	4-P	.20	273	86.6	38.89	15.23	4.36	6.35	7.9	-10	44.5	.....	23.0	
B	7-P	.20	241	111.5	37.26	14.21	4.23	6.38	18.0	0	44.0	.....	23.0	
C	12-P	.20	264	92.6	40.55	13.63	4.33	6.39	2.0	-10	41.0	Ox.P.	22.0	
C	13-P	.15	241	112.5	39.08	12.29	4.63	6.33	7.6	30	42.0	L.F.F.	22.0	
D	23-B	.25	335	53.2	38.17	13.69	4.19	6.64	4.4	-10	42.0	Cn.	23.5	
D	24-P	.15	222	128.6	37.38	13.27	4.40	6.76	13.7	0	43.0	Cd.	22.5	
D	26-P	.20	275	85.9	37.63	13.59	4.32	6.43	17.2	0	42.0	Cn.	23.5	
E	5-P	.20	262	93.5	38.22	12.32	3.96	6.38	10.5	0	43.0	L.F.F.	22.5	
F	9-P	.20	303	69.2	39.04	13.93	4.03	6.39	30.0	-10	44.5	.....	22.5	
G	10-P	.20	270	89.5	37.85	13.45	4.57	6.42	1.1	270	40.0	V.Un.	21.0	
H	11-P	.17	305	68.4	39.56	13.70	4.41	6.40	3.2	-10	42.0	Un.	22.5	
I	14-P	.15	212	141.1	39.61	12.16	4.13	6.35	8.8	190	41.0	Un.	21.0	
J	15-P	.15	318	61.9	39.16	13.73	4.00	6.39	0.5	0	43.5	T.S.	22.5	
K	16-P	.20	304	68.2	40.19	16.97	3.66	6.37	51.2	-10	38.0	Cy.O.I.	22.0	
L	17-P	.15	244	108.7	37.46	13.92	4.52	6.56	9.5	60	38.5	O.I.U.n.	22.5	
M	18-P	.15	264	94.3	38.25	14.00	4.08	6.40	1.7	0	39.0	Un.	22.5	
N	19-P	.20	228	125.3	39.35	13.57	4.10	6.41	1.0	0	41.0	Un.	22.0	
O	20-P	.20	231	119.1	38.51	13.66	4.08	6.74	2.4	0	42.0	L.F.	22.0	
P	21-P	.20	413	24.2	38.99	13.47	4.02	6.40	0.3	-10	43.5	.....	22.5	
Q	22-P	.15	225	125.1	38.85	15.31	4.10	6.40	19.2	20	44.0	.....	22.5	

TABLE 1--(Continued)

Plant	Sample	Pint price	Net wt.	Over-run case.	Total solids	Butter fat	Protein N x 6.38	pH	Bacteria per ml.		Score	Criticism*	Score	Criticism**	
									%	%					
R	26-P	\$	287	79.1	38.67	13.35	4.16	6.38	5.2	0	42.0	SL.C.K.	23.5		
B	27-B	\$	465	9.8	38.34	13.23	4.16	6.38	77.0	-10	43.5	SL.C.K.	24.0	H.	
B	28-B	\$	403	25.9	38.97	13.68	4.20	6.43	154.0	-75.0	43.5	SL.C.K.	24.0	SL.H.	
B	30-B	\$	460	11.2	38.45	13.24	4.21	6.37	125.0	30	44.0	L.F.P.	23.5	Cr.	
B	32-B	\$	295	73.7	39.84	13.52	4.24	6.35	43.0	-10	43.5	SL.C.K.	24.0	SL.H.	
B	37-B	\$	20	360	42.6	39.17	14.32	6.42	6.43	29.0	-10	43.5	SL.C.K.	23.5	Icy.
A	31-B	\$	25	339	50.8	37.21	13.54	6.38	6.43	26.0	-10	44.0	I.S.	23.5	Icy.
A	33-B	\$	25	409	24.9	37.42	13.61	6.28	6.39	3.5	-10	44.0	I.S.	24.0	SL.H.
D	35-B	\$	20	330	53.4	38.23	13.34	6.20	6.65	32.0	-10	44.5	L.F.P.	23.5	SL.FI.Cr.
O	36-B	\$	20	219	131.3	38.48	13.88	4.46	6.41	4.5	50	40.0	U.n.	23.0	Fl.Cr.
Q	34-P	\$	15	246	109.2	38.30	12.94	4.31	6.40	34.0	30	44.5	L.F.P.	23.5	Fl.
S	28-P	\$	15	251	104.3	37.86	13.10	4.13	6.45	120.0	10	42.5	C. or P.	22.5	Icy.Cr.
Average all samples		V.	19.4	298.0	80.0	38.64	13.65	4.24	6.43		42.5			22.8	
Average 15¢ samples		V.	257.8	104.0		38.56	13.40	4.25	6.43		42.1			22.5	
Average 20¢ samples		V.	290.1	81.9		38.76	13.85	4.24	6.43		42.4			22.7	
Average 25¢ samples		V.	373.2	40.3		38.39	13.63	4.24	6.42		43.3			23.7	

\* Flavor criticism key:

Un.—Unnatural.  
V.—Very.  
L.F.—Lacks flavor.  
L.F.F.—Lacks fine flavor.  
O.I.—Old ingredient.  
P.—Powder.  
Ox.P.—Oxidized powder,

C.—Condensed.  
Sl.—Slight.  
Caramel.  
Cooked.  
Cow.  
Too sweet.  
L.S.—Lacks sweetness,

\*\* Body criticism key:

Fl.—Fluffy.  
H.—Heavy.  
Icy.—Icy.  
Slight.  
Course.

relationship was found on the average for all samples; that is, the average weight was higher for each increase in price level. When converted to a basis of the net weight of ice cream purchased for a certain price, that is, twenty cents, we find the advantage to the consumer decidedly in favor of the ice cream selling at the lowest price level, with the twenty and twenty-five cent pints about equal, but with a slight advantage (8.4 grams) for the higher priced pints.

The per cent overrun as calculated includes the extremes of 9.8 and 141.1 with an average of 80.0. The minimum overrun for all samples was found in the fountain-dipped package group; the maximum for this group was 73.7 per cent. The maximum overrun for all samples occurred in the factory-filled package group, while the minimum overrun for this group is 24.2 per cent. The highest overrun occurs in the lowest price level group with a minimum overrun for this group of 25.9 per cent.

In a somewhat similar comparison of ice cream purchased in metropolitan Chicago, Grumbine and Halliday (1) found a range in weight of pint samples from 248.8 grams to 480.2 grams and the overrun to include the extremes of 14.1 per cent and 143.0 per cent.

If 6.4 to 6.6 is accepted as the normal pH of ice cream mix made from fresh ingredients as proposed by Sommer (2), we may assume two of the samples obtained were made from slightly neutralized mix.

A study of the total solids content of these samples showed a range from 37.21 per cent to 40.19 per cent with no significant difference in this characteristic between bulk and factory-filled packages. The butterfat content varied from 12.16 per cent to 16.97 per cent with both of these extremes occurring in the factory-filled package group. On the average there was a slightly higher percentage of butterfat in the factory-filled pints and in the higher-price-range pints. In making this comparison it should be borne in mind that the state law (3) specifies at least 14 per cent of butterfat for vanilla ice cream, with this exception: "There shall be allowed a tolerance of one-half per cent, provided the one-half per cent is not constantly below the standard." The percentage of samples containing less than the legal percentage of fat was relatively uniform for all groups of samples; this was 78.4 per cent for all samples, 81.8 per cent and 80.8 per cent respectively for bulk and package samples, and 70.6 per cent for the twenty-cent-pint group. On the basis of the 13.5 per cent tolerance, 37.8 per cent of all samples failed to comply as did 36.4 per cent and 42.3 per cent, respectively, of the bulk and package samples and 35.3 per cent of the twenty-cent-pint group.

In making a comparison of these samples on the basis of calculated calorific value received for an expenditure of twenty cents (table 2), the average for all samples was 696.2 but with the wide range of 489.2 to 1205.1. The bulk samples showed the widest variation in this respect of any group, while the lowest price group was second in variation.

In examination of the results of bacterial analysis of these samples, the total count of six samples was above 50,000; four of these were fountain-dipped packages and two factory filled. In the less-than-10,000-total-count group there were eighteen samples; two of these were fountain-dipped pints and sixteen factory-filled pints. The three samples with the highest total count were all fountain-dipped pints, one at 1½ million and the other two at approximately 125 thousand. Only one factory-filled pint occurred in the group with a total count of more than 100,000.

TABLE 2  
*Quantities purchased for twenty cents*

	Total solids	Butter-fat	Protein (N × 6.38)	Carbohydrates (calculated)	Calories (calculated)
All samples (average) .....	gms.	gms.	gms.	gms.	
120.3	42.4	13.2	65.4	696.2	
15-cent samples (average) .....	132.4	46.0	12.8	71.1	761.6
20-cent samples (average) .....	112.5	40.1	12.3	61.7	656.6
25-cent samples (average) .....	114.5	40.6	12.6	61.2	660.7
Factory-filled pints (average) .....	115.8	41.0	11.8	62.2	668.2
Fountain-dipped pints (average) .....	130.9	42.8	14.5	73.0	762.6

The three samples with the highest coli count were in the fountain-filled group, one in the lowest price level and two in the twenty-cent-pint group. Two of these three samples, one of which was in the lowest price level, were highest and second highest of all samples in total count. The sample containing the third highest number of coli showed a total count of 32,000. In the four cases of factory-filled packages in the less-than-10,000-total-count group the number of coli was 270, 200, 190, and 50 per milliliter, indicating that the coli content of these samples did not originate with the dispenser.

The flavor and body scores of the samples in the fifteen- and twenty-cent-pint groups show no significant difference. The twenty-five cent pints and the fountain-filled pints averaged approximately one point higher in body score and in flavor score than the average for all samples.

When the results of the analyses made were computed to the basis of product received for a definite expenditure (table 2), the advantage was with the lowest-priced group in all comparisons made where different price level groups were the basis for the comparison. In a similar comparison it was indicated that the purchaser of the fountain-dipped package receives an advantage as compared to the purchaser of the factory-filled package when these two groups are the basis for comparison.

#### CONCLUSIONS

This preliminary study of a limited number of pint samples of vanilla ice cream at all price levels available to the consumer in the trade territory

studied does not indicate the reason for the difference in price level when a comparison is made on the basis of the following: net weight of ice cream obtained; calculated overrun in per cent; composition including butterfat, total solids, protein and calculated carbohydrate; bacteria count of either total or colon type organisms; calorific value purchased for a certain expenditure, or quality as determined by organoleptic examination.

It is recognized that this is not an all-inclusive study of a problem of this character but it is believed that studies such as this carried out in a market at intervals of six months or one year would tend to bring to the consumer a more uniform product and tend to aid in establishing a sound basis for differences in price per unit quantity of ice cream purchased.

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# THE VALUE OF SODIUM METAPHOSPHATE IN DETERGENT MIXTURES IN THE CLEANING OF MILKING MACHINES\*

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The process of cleaning the milking machine has claimed the attention of the dairy sanitarians ever since the advent of this device. Although numerous publications have appeared from time to time recommending various methods of cleaning and sterilizing, and although these methods can be applied with considerable success, the fact remains that milking machines are still serious foci of contamination in market milk. The mechanical structure of the machine, even though marked simplifications have been affected, makes the cleaning and disinfecting procedure difficult when compared to the equipment necessary for hand milking. This does not mean that the equipment is really difficult to clean, but it does mean that the method of care is different and peculiar to this equipment. The milking machine is such a valuable adjunct to the dairy farm that the more or less insanitary conditions of these machines that prevails should be rectified by better methods of cleaning so that their use will not be discredited.

Not only may the dirty milking machines be an important source of bacterial contamination, but they may also be a reservoir of heat-resistant bacteria, which are responsible for the high count occasionally obtained in properly pasteurized milk. The role of milking machines as a source of heat-resistant bacteria is not unknown to the dairy sanitarian. Many times the dairy plants are accused of insanitary conditions or carelessness when most of the trouble lies with the producers who have been careless in the handling of their milking machines.

## EXPERIMENTAL

In the spring of 1938, a large dairy in Michigan was having considerable trouble with high counts in its pasteurized milk. On several occasions samples were collected on three different routes from producers using milking machines. These machines had received little attention from the milk inspector so they represented conditions which may prevail under lax inspection. The producer samples were tested by the standard plating procedure. Each sample was then pasteurized and another plating made to

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determine the reduction affected by this heating. The data are presented in table 1. The data show that a large percentage of the bacteria in these milks were resistant to pasteurization temperatures. Not only were the counts of raw milk high, but the percentage of reduction in count by pasteurization was unsatisfactory. Only 6 out of 23 samples showed reductions of 95 to 100 per cent. The picture presented by the bacteria counts of these producer samples is not unknown where inspections are lax.

TABLE 1  
*Bacteria counts of samples from producers using milking machines where field inspection was inadequate*

Sample No.	Bacteria count of milk		Per cent reduction
	Raw	Pasteurized	
1 .....	40,000	26,000	35.0
2 .....	2,900,000	550,000	81.0
3 .....	39,000	25,000	35.9
4 .....	87,000	32,000	63.2
5 .....	32,000	40,000	0.0
6 .....	70,000	17,000	75.7
7 .....	3,250,000	470,000	85.5
8 .....	3,900,000	1,120,000	71.3
9 .....	96,000	97,000	0.0
10 .....	121,000	65,000	46.6
11 .....	1,400,000	166,000	88.1
12 .....	172,000	5,000	97.2
13 .....	4,300,000	274,000	93.6
14 .....	80,000	20,000	75.0
15 .....	940,000	26,000	97.1
16 .....	380,000	10,000	97.4
17 .....	625,000	21,000	96.7
18 .....	345,000	195,000	46.7
19 .....	1,200,000	82,000	93.2
20 .....	175,000	90,000	48.6
21 .....	550,000	18,000	96.7
22 .....	760,000	13,000	98.3
23 .....	2,100,000	131,000	93.8
Average .....	1,024,000	152,000	
Geom. mean .....	374,000	21,000	94.3

At the same time that the above-mentioned samples were collected, examination was made of producer routes under inspection by a city dairy inspector and a field man employed by the dairy. Samples were collected from producers using milking machines. The samples were handled in exactly the same manner as before. The results are presented in table 2. The results show that the bacteria counts were reduced from 95 to 100 per cent in 9 out of 16 samples tested. With the exception of two producers, Nos. 2 and 6, there was little evidence of heat-resistant organisms. The two samples cited showed the presence of heat-resistant bacteria as indicated by the high pasteurized milk counts of 19,000 and 58,000 per ml., respectively.

TABLE 2

*Bacteria counts of samples from producers using milking machines where field inspection was adequate*

Sample No.	Bacteria count of milk		Per cent reduction
	Raw	Pasteurized	
1 .....	34,000	1,000	97.1
2 .....	285,000	19,000	93.3
3 .....	4,000	100	97.5
4 .....	17,000	500	97.1
5 .....	82,000	7,000	91.5
6 .....	630,000	58,000	90.8
7 .....	655,000	2,000	99.7
8 .....	17,000	350	97.9
9 .....	170,000	6,000	96.5
10 .....	6,500,000	2,000	99.9
11 .....	38,000	4,000	89.5
12 .....	40,000	7,000	82.5
13 .....	75,000	9,000	88.0
14 .....	35,000	9,000	74.3
15 .....	190,000	3,000	98.4
16 .....	575,000	400	99.9
Average .....	584,000	8,000	
Geom. mean .....	104,000	2,700	97.4

The results of these two series of tests, which are representative of others, made on these milk routes indicate that either the methods of cleaning or disinfecting the machines were faulty, or the performance of the same was not carried out successfully. From observations by the inspectors criticisms might be made rightfully that both methods and the application of the same were improper. The tests show that regular inspection is necessary and that even then milking machines may still produce an unsatisfactory supply of milk for pasteurization.

There is a dearth of information on methods of cleaning milking machines. Most publications place emphasis on the sanitation. The most common practice in cleaning consists of flushing machines with cold water after each milking and then placing the tubes on the racks for either alkali or chlorine treatment. Under these conditions machines are washed once a week with hot water and a detergent. This procedure is very poor operating practice.

A satisfactory method of cleaning consists in flushing with cold water immediately after milking, then with warm water and a detergent, and then placing the tubes on the racks for disinfection. This procedure does produce satisfactory results, but unfortunately this method is seldom practiced and even where it is used the method is not carried out with sufficient thoroughness to render the equipment free of milk wastes and lime deposits.

In the routine examination of milking machines, considerable accumulations of calcium phosphate or milkstone in the rubber tubing and the inner surfaces of the milking machines have been found irrespective of the

methods of cleaning, where the procedure was not thorough. These accumulations, as pointed out in the literature, are produced largely by the improper removal of the milk film, but the deposits of lime from the use of calcium hypochlorite and the salts from hard waters also play an important role.

The presence of milkstone is generally accepted as an indication of improper cleaning. Machines showing the presence of this substance are considered as insanitary, but in spite of continuous and frequent inspection it is commonly found.

It is the experience of the senior author that no chemical disinfection on the market today will function when the surface to be disinfected is covered with deposits such as occur in the rubber tubing of the milking machine. Neither lye nor chlorine has any penetrating power, as demonstrated by Mallmann and Chandler (1). They show the need of cleanliness as a necessary preliminary to disinfection. No disinfection can act economically or efficiently in the presence of excessive amounts of suspended materials or film providing the bacteria are embedded.

The problem of producing a better quality milk with milking machines appears to lie not in the development of a better sanitizer but in the development of better means of cleaning applicable to farm use and practice. It would seem that in addition to obtaining better disinfection by presenting a clean surface to the disinfectant, the proper cleaning of equipment would present clean surfaces to which the bacteria could not remain attached.

No data are available on milking machines to demonstrate the comparative effects of clean surfaces as compared to milkstone-covered surfaces in producing a low count milk. Accordingly, tests were made to measure the value of clean milking machine equipment in reducing bacteria counts. Arrangements were made with a local dairy to conduct tests in cooperation with some of their producers who were using milking machines. The samples were collected for a period of two weeks prior to cleaning the machines to obtain approximately average conditions with machines receiving the usual cleaning. After the collecting of these samples all the machines were carefully cleaned by the inspectors to be sure that they were free from milkstone. No change was made in the method of cleaning employed by the producer. Samples were collected for a period of three weeks following the cleaning of the machines by the inspectors. The results are given in table 3. It will be observed that a marked fall occurred in the geometrical count after cleaning. This reduction persisted for the entire period of three weeks for which the tests on the cleaned equipment were made.

In compiling the data before and after cleaning, two variables, cleaning and temperature, must be considered. The influence of temperature can

TABLE 3  
*The effect of cleaning milking machines on the bacteria count of the milk*

Time of collection	Geometrical mean		
	No. of samples	Plate count	Microscopic clump count
Before cleaning .....	37	490,000	165,000
After cleaning .....	78	231,000	99,000

be eliminated by the fact that warmer weather occurred after cleaning, as the tests were started in late April and were discontinued early in June. As a matter of fact, if the temperature before and after cleaning had been constant the reduction would have been more pronounced than that reported. Thus, the reduction in bacteria count of the milk from the cleaned machines is due to the cleanliness of the machines themselves.

The use of tri-sodium phosphate, sodium metasilicate, and lye under certain conditions causes a formation of calcium phosphate or milkstone. This formation acted as a protective layer to the bacteria against chemical disinfection and also as a reservoir for the growth of bacteria. As demonstrated earlier in this paper, unclean machines are frequently sources of heat-resistant bacteria. If the formation of milkstone could be prevented it would be possible to produce low count milk with a minimum of heat-resistant bacteria. Schwartz and Gilmore (2) demonstrate that the addition of sodium metaphosphate to a detergent mixture used for mechanical dishwashing produced better results and eliminated the deposition of scale on the surface of the dishes and the equipment. Gilmore (3) states, "Sodium metaphosphate, although not a detergent, by forming soluble complex ions with the ions of calcium and magnesium in alkaline solutions, prevents completely the formation of insoluble alkaline salts and soaps that constitute the deposit formed in ordinary processed water."

To determine the value of sodium metaphosphate as a means of preventing the formation of milkstone, two sets of milking machines were tested in parallel. One set was flushed daily with a detergent mixture containing 20 per cent sodium metaphosphate, and the other set was cleaned in the same manner as the producer had used prior to the test. Both sets of machines were carefully cleaned at the start of the experiment. Samples were collected from each producer from time to time over a period of three weeks. The data for these experiments are presented in table 4. The data show that reductions in bacteria count result whether a sodium metaphosphate detergent mixture or other means of cleaning was employed. The data do not show that the sodium metaphosphate detergent mixture has any advantage from the standpoint of bacteria reduction over other methods of cleaning during a period of three weeks following a thorough cleaning of all of the machines.

The fact that all of the machines were cleaned at the start of the experiment and the producers were made aware of the fact that their cleaning

TABLE 4  
*The effect of cleaning milking machines with a detergent mixture containing sodium metaphosphate*

Time of collection	Duration of tests	Geometrical mean		
		No. of samples	Plate count.	Microscopic clump count
Sodium metaphosphate used				
Before cleaning .....	2 wks.	41	193,000	364,000
After cleaning .....	3 wks.	71	172,000	25,000
No sodium metaphosphate used				
Before cleaning .....	2 wks.	37	490,000	165,000
After cleaning .....	3 wks.	78	231,000	99,000

operations were unsatisfactory would likely cause them to be more careful. As a result, during the three weeks period of the tests both groups kept their machines in a clean condition, in spite of rather than because of, the type of cleaner used. It was quite evident that the amount of accumulation in the tubes would be slight in three weeks, and a longer period would be necessary to demonstrate the difference between a clean machine and one containing milkstone deposits.

The experiments were accordingly continued over a longer period of time. At the end of five and eleven months the monthly routine samples were checked for each group. These results are presented in table 5. It will be observed that the geometrical mean microscopic count showed a marked reduction in number of bacteria in favor of the sodium metaphosphate detergent mixture at the end of both five and eleven months periods. These experiments show that the introduction of sodium metaphosphate into the detergent mixture tends to keep the bacteria count at a relatively lower level than the cleaners used which lack this material.

An examination of the physical appearance of the milking machines showed a very marked difference between the machines cleaned with the sodium metaphosphate detergent mixture and those cleaned with other

TABLE 5  
*The effect of cleaning milking machines with a detergent containing sodium metaphosphate*

Detergent contained	No. of producers	Duration of test	Geometrical mean
			Microscopic clump count
Sodium metaphosphate .....	7	5 months	110,000
No sodium metaphosphate .....	7	5 months	219,000
Sodium metaphosphate .....	7	11 months	77,000
No sodium metaphosphate .....	7	11 months	113,000

cleaners. The machines were examined nine months after the start of the experiment. At that time every one of the machines cleaned with the sodium metaphosphate detergent mixture was free from milkstone on both the metal and rubber surfaces, and the machines were pronounced perfectly clean. On the other hand, many of the control machines were heavily coated with milkstone, particularly in the rubber hose lines.

#### CONCLUSION

The experiments cited show that the use of a detergent mixture containing sodium metaphosphate prevented the formation of milkstone on both the metal and rubber parts of the milking machines. The use of milking machines kept free of milkstone deposits made it possible to produce a lower count milk than was produced with the machines containing milkstone.

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# A REVIEW OF OXIDATION IN MILK AND MILK PRODUCTS AS RELATED TO FLAVOR<sup>1</sup>

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## I. INTRODUCTION

Tallowy, oily and fishy flavors, which are considered to be caused by oxidation, have been observed in storage butter since the early days of the industry. The objectionable nature of these off-flavors caused losses which drew the attention of investigators to the problem of their prevention. Early investigations showed that air (152, 192, 194, 318), light (152, 192, 194, 305, 318, 383) contamination by metals (11, 164, 209, 230, 392), acidity of the cream (131, 241, 242, 274, 275, 322) salt content of the butter (131), and storage temperature (131, 192, 194) all are factors related to the development of these flavor defects. Later work showed that several other factors may promote the oxidative changes in butter which affect flavor.

More recently work on oxidation in milk products other than butter has been stimulated by investigations of the effects of metals on these products. Examples of this type of investigation are the work of Hunziker and associates (180, 181, 182) which showed that certain metals, particularly copper and iron, dissolve in the milk brought into contact with their surfaces and cause undesirable flavors; the work of Rice (288) which showed that tallowiness in sweetened condensed milk may be caused by copper contamination resulting from contact of the milk with copper in vacuum pans; and, the report of Okuyama (260) which showed that copper contamination of milk during processing, rather than contact of the milk with the bottle cap, was causing so-called cappy flavor in the milks from four milk plants under the control of his laboratory.

Examination of the literature available at the present time shows that many of the factors which favor oxidative changes in butter are concerned also with the production of off-flavors in milk, dried milk, ice cream, and condensed milk. Since a given factor may produce oxidative changes in more than one dairy product, each factor, as it relates to various dairy products, will be discussed separately.

## II. EFFECT OF MICRO-ORGANISMS

**Butter**—In 1894 Von Klecki (383), studying rancidity and the acid number of butter, observed that bacteria caused an increase in acidity.

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Probably this increase was not related to oxidative changes because later work by Sayer and associates (322), Rogers and co-workers (306, 307), and Stokoe (343) shows that micro-organisms are not associated with the development of oily and tallowy flavors in butter. However, Ritter and Christen (299) have found that active cultures of certain alkali-producing bacteria retard the development of fishiness in butter, perhaps by retarding or reducing acidity (see Sect. VII).

*Milk and Cream*—The relation of micro-organisms to the development of off-flavors in the fluid products, milk, cream, and ice cream, is quite different. Kende (198) has shown that the addition of a culture of bacteria which grew well at temperatures below 10° C. prevented the development of oxidized flavor in milk. Kertesz (200) isolated this species and gave it the name *Reducto-bacterium Frigidum neutrale*. A preparation of this culture has been made in dry form by drying milk which has been sterilized and inoculated. According to Ritter and Christen (296) the dry culture is effective in preventing oxidized flavor when added to milk or cream in a proportion of 1 to 25,000. They examined this preparation and found that it does not reduce methylene blue but does reduce 2, 6-dichlorophenol-indophenol; and that the active material can be obtained from milk sugar by extraction with alcohol or ether. They were able to isolate 5 to 7 per cent of hydroquinone from the dry material and considered this substance to be the active antioxidant. Kertesz (201) replied to Ritter and Christen stating that hydroquinone is known to be a product of the metabolism of certain bacteria and pointing out that it has no harmful physiological effects. He stated also that the reducing bacteria are not of a single type, but are embraced within a group whose individual types vary in the production of reducing compounds.

Tracy (363) states that oxidized flavor develops more rapidly at 4° than at 20° C. and Thurston and Olson (361) noticed an oxidized flavor in milk which had been stored for several days at 38° F. and which showed little bacterial growth, whereas a duplicate sample of the same milk stored at 52° F. showed considerable bacterial growth but no oxidized flavor. Roland, Sorensen, and Whitaker (310) found that bacterial counts of milk which showed oxidized flavor were generally lower than the counts of milk free from this defect. Tracy, Ramsey and Ruehe (366) extended information on the subject when they reported that oxidized flavor was less likely to occur in milk incubated at 68° to 90° F. for 1 to 6 hours before cooling and holding at 40° F. than was the case when milk was stored at 40° F. immediately without incubation. Cream which had a tendency to develop oxidized flavor without incubation, when incubated showed a higher average score of about 3.5 points. They also found that the addition of living yeast cells to milk retarded the development of tallowiness, whereas the addition of dead cells had no effect.

Esselen (98) found that the growth of bacteria significantly retards the oxidation of ascorbic acid, especially when cultured in media containing fermentable carbohydrates.

There is considerable evidence to show that the retarding effect of the growth of micro-organisms on the development of oxidized flavor in milk and cream accompanies and is usually parallel with a lowering of the oxidation-reduction potential. This has been shown by Kende (198) and by Tracy and co-workers (366). The work of Thornton and co-workers (354, 354a) and of Fay and Aikins (1, 102) on the methylene blue reduction test shows that the growth of micro-organisms in milk causes a lowering of its oxidation-reduction potential. This effect appears to be the result of the removal of dissolved oxygen by bacterial growth and the influence of natural reducing substances in the milk. The effect of absorbed oxygen was shown in an interesting way by Jackson (189) who found that milk drawn anaerobically from the udder of the cow reduced methylene blue almost instantaneously, whereas the same milk exposed to the air under normal conditions usually required more than 10 hours to reduce the dye. Strynadka and Thornton (344) found that abnormal udder conditions responsible for milk of high leucocyte content also were responsible for abnormally high concentrations of reducing substances in milk, although they observed that in common practice leucocytes are rarely, if ever, the main or significant influence in the reduction of methylene blue in milk. Davis (79) showed reduction of several dyes in ripened cheddar cheese, apparently brought about by the growth of bacteria.

### III. EFFECT OF METALS

*Iron and Copper Contamination*—Weigmann (392) was one of the first to observe the catalytic effect of iron in the oxidation of butter fat. In 1891 he attributed oily flavor in butter to iron which contaminated the cream kept in poorly tinned vessels for creaming. Marcus and Huyge (230) noted a bitter, astringent taste in the butter from milk kept in rusted iron cans, whereas milk held in non-rusted cans was of good quality. They found that cream having a normal iron content of 0.005 parts per 1,000 would increase to 0.240 parts after 22 hours contact with a rusted can, and to 0.270 parts per thousand after 46 hours. The butter from cream containing 0.240 parts per thousand contained 0.080 parts of iron per thousand of cream. Butter made from cream containing 0.270 parts per thousand contained 0.134. They concluded that the cream coming in contact with iron rust formed a lactate from the iron oxide and that the lactate was responsible for the bitter taste observed. The solvent action was found to be especially pronounced in cream of high acidity. Hoft (164) made butter from cream which stood for 22 hours after the addition of 2 to 33 parts per million of ferrous ammonium sulfate. In the majority of the eight butters on which the report was based,

an oily metallic taste was observed. He made the significant observation that small amounts of iron acting for a long time had more effect than larger quantities acting for a shorter time.

The effect of washing butter with water containing iron has been studied. Kooper (209) using a suspension of finely divided metallic iron in the water reported no effect on the butter washed with water containing iron in quantities up to 36 mg. of iron per liter. He believed that changes which may take place are caused by other substances in the water together with the iron and suggested that these substances probably are hydrogen sulfide or nitrous acid. The addition of rusted nails or pulverized iron rust to cream before ripening caused oily and metallic flavors in the butter. Another investigator (11) reported that 9 to 15 mg. of soluble iron per liter of wash water caused oily and metallic flavors in the butter and that butter washed with water from which iron had been removed by oxidation and filtration did not show off-flavors. However, this investigator agrees with Kooper (209) in regard to the production of oily and metallic flavors by cream held in rusted vessels. Our present knowledge would lead us to believe that solubility is paramount in this connection.

Rogers and associates (307) added copper and ferrous iron in the form of lactates or sulfates to cream and observed that distinct oily, metallic or fishy flavors developed in the butter after churning. The copper caused more intense flavors than did the iron.

In 1917 Hunziker and Hosman (183) found that copper, as well as iron, catalyzed the development of tallowy flavor in butter. Either an iron nail or a copper wire imbedded in the butter caused bleaching and tallowiness. The addition of colloidal hydroxides of iron and of copper in minute quantities to butter (4 drops per 180 grams of butter) was tried with the result that the copper-contaminated sample became tallowy in 8 days, whereas the iron-contaminated sample did not show this defect after two months in storage at 32° F. Pure butter fat emulsified with casein and a slight excess of alkali showed slight tallowiness and bleaching after 5 days at room temperature in the presence of iron, copper, brass, or German silver. These samples were intensely tallowy and bleached after 28 days. Samples containing nickel and tin, which were normal after 5 days, had turned slightly tallowy without bleaching; and the butter fat of normal lactose content emulsified without acid, alkali or metal addition remained normal. The iron-contaminated sample containing alkali became fishy. In all cases of bleaching and tallowiness observed by these workers the defects appeared first at the surface of the butter and developed inward. Later Hunziker (178) pointed out that the use of copper or German silver equipment in the processing of cream when the cream also was exposed to the action of air and light yielded butter which would develop a metallic, oily or fishy flavor.

Singleton (331) states that the great majority of creamy butter in New Zealand has an iron content well below the maximum desirable figure of 1.5

p.p.m. but that a much higher proportion of the samples had a copper content exceeding the desirable maximum of 0.2 p.p.m.

Miwa (244) reports that the copper content of dried milks vary with brands and that the maximum content was 0.03 mg. per 10 grams while the minimum was 0.02 mg. In addition he reported that Japanese dried milk contained more copper than other foreign makes. Hunziker (177) observed that the metallic flavor often found in sweetened condensed milk was due to the formation of copper oxide in the dome of the vacuum pan, and that when the copper, both in the pan and in its dome, were kept clean, bright, and shining, the product was practically free from a metallic taste.

Emery and Henley (97) found that lard, corn oil, and cottonseed oil developed rancidity (oxidative) when stored in copper or iron containers and that lacquering the containers prevented this development. Clavel (49) warned that metals such as iron which dissolved when fats are stored in metal tanks or containers catalyze the reaction causing rancidity.

In 1905 Golding and Feilman (125) reported that milk passed over a partially detinned cooler acquired a metallic flavor after about 18 hours. No doubt the so-called metallic flavor was identical with that referred to as oxidized flavor at present for the catalytic effect is indicated by the fact that 18 hours intervened between contamination and development of the flavor. Little attention was given to the oxy-catalytic effect of copper in milk, however, until attention was focused on the effects of metals on milk and of milk on metals by the work of Hunziker (180) in 1925. Hunziker pointed out that there were then many cases on record where the use of copper-coil surface coolers in milk plants had caused the milk to develop a disagreeable metallic flavor. In most of these cases the tin coating on the copper was defective and retinning invariably prevented further trouble. Hunziker cites numerous cases in which copper-bearing equipment had been known to cause development of undesirable flavor in milk. He reported also that iron contamination of milk produced similar effects although to a lesser degree. In 1927 Okuyama (260) showed that a cappy or pulpy flavor, noticed at four different milk plants, was caused by copper contamination.

The effect of copper contamination on the flavor of milk brought to attention the natural copper content of milk as a possible cause of oxidized flavor. In 1922, Supplee and Bellis (346) reported uncontaminated milk to contain 0.04 mg. of copper per 100 grams. Later work has indicated this figure to be too high although workers are not in agreement as to the amount of copper present. One group of workers (93, 116, 244) have found the average copper content of milk to be about 0.3 mg. per liter. Other workers (62, 210, 211, 412) report the natural copper content of milk to be 0.14 to 0.17 mg. per liter. These results indicate the need for accurate and reliable methods for the determination of copper in minute amounts.

Likewise, results vary as to the amounts of iron found naturally in milk. Davies (88) reported the iron content of uncontaminated milk to vary

between 1.18 and 2.32 p.p.m., whereas Dahlberg and Carpenter (63) reported an average of 0.379 mg. per kg. Over the course of a year, Krauss and Washburn (211) reported the iron content of milk to vary between 0.34 and 0.43 mg. per liter. Guthrie and Brueckner (142) found that certain cow's milk developed oxidized flavor without metallic contamination.

Evidently as better methods become available, better agreement on the mineral content of milk will be obtained. Echave (93) obtained a slight increase in the copper content of milk produced on feeds high in copper. However, Liebscher (219) was unable to increase the copper content of milk by feeding beet tops treated with copper. Rice and Miscall (289), in 1923, were two of the earliest workers to determine experimentally the amount of corrosion of copper by milk. They used a procedure in which copper strips were immersed in milk under various conditions and determined the amount of corrosion by loss of weight. Many workers have used this procedure with minor variations.

Temperature was one of the first factors shown to influence the rate of corrosion. Rice and Miscall showed that less copper was dissolved at boiling temperature than at room temperature. Whitfield *et al.* (402) reported greater corrosion at 144° F. than at 60° F. Gebhardt and Sommer (117, 118, 119) showed that the solubility of copper increased up to 158° F. and then decreased. Trebler *et al.* (369) found greater corrosion of copper, nickel, and nickel-silver in milk at 140° F. than at 158° F. Miscall and co-workers (243) found that the dissolving power of milk increased up to 140–145° F. and then decreased. Quam (283) working with copper in milk at temperatures ranging in 9° intervals between 86° and 212° F. reported the maximum corrosion at 185–194° F.

The presence of oxygen has been shown to increase the rate of copper corrosion, whereas the presence of carbon dioxide decreased it (117, 118, 119, 243, 289, 402). Copper with an oxidized surface shows an increased amount of corrosion (289). Henderson and Roadhouse (155) reported lower rates of corrosion for copper in alloys containing tin and zinc.

Many workers (104, 119, 145, 214, 240, 282, 293, 369, 396, 402) have observed high corrosion rates for nickel exposed to the action of milk and an oxidized flavor has been observed in one instance (145), a metallic flavor in another (402) and an off-flavor in still another instance (293). In the last case the off-flavor was not tallowy when compared to the copper-induced flavor.

Aluminum (104, 119, 144, 145, 181, 213, 282, 293, 396, 402) has been shown to withstand corrosion by milk and does not give off-flavors. However, aluminum is readily corroded by alkali washing powders (181).

Chromium-nickel steel, stainless steel, and Allegheny metal (119, 144, 181, 182, 281, 369, 396, 402) withstand corrosion by milk and produce no off-flavors in the milk.

Galvanic effects can occur wherever dissimilar metals are coupled in hot, aerated pasteurized milk. Likewise, galvanic corrosion can occur solely as a result of a difference in temperature of identical metal surfaces exposed to a given solution. These effects are well known, and certain metal couplings are avoided. Wesley, Trebler, and LaQue (396) prepared a cell with a nickel electrode in milk and studied the effect of temperature variations. They concluded that the temperature effect is of little importance under pasteurization conditions, and that in this instance the galvanic effect of differential aeration can be of greater importance than that of temperature difference.

Fink and Rohrman (104) have demonstrated that nickel may replace copper in solution during pasteurization and that the resulting pasteurized milk may be lower in copper content than the milk prior to pasteurization.

Ellenberger and White (94) observed a metallic flavor in cream pasteurized in contact with copper and stored for six months at 0° F. Guthrie (141) observed that metallic flavors were caused by factors other than the direct contact of the cream with the metal. He observed that cream only slightly metallic in flavor yielded buttermilk with a strong metallic flavor even when churned in a glass churn. Rogers, Berg, Potteiger, and Davis (307) studied the factors influencing the change of flavor in storage butter and observed that ferrous sulfate or ferrous lactate added to cream prior to churning caused a distinct off-flavor described as oily, metallic or fishy. Copper sulfate and copper lactate produced these results much more intensely than the ferrous salts. Hunziker and Hosman (183) and Hunziker (178) verified the findings that metallic and oily flavors were related to either iron or copper contamination. They likewise observed that copper was more active than iron in bringing about this defect.

In addition to oily, metallic, and tallowy flavors of butter, fishy flavor appears to be closely correlated with iron and copper contamination. In 1923 Sommer (335) reported before the World's Dairy Congress that trimethylamine is the immediate cause of fishiness in butter and that it is produced mainly, if not entirely, by the chemical decomposition of lecithin. In a discussion of Sommer's paper Hunziker characterized tallowiness and fishiness as closely related flavors. He regarded both as arising from the same combination of factors with the exception of the acid reaction which accompanies fishy flavor.

The development of fishiness in butter (193) like that of tallowy flavor in cream (298) appears to be retarded or reduced by high temperature pasteurization. Likewise bacteria which reduce the oxygen content of butter (193) or cream (297, 298) retard or inhibit these flavor defects.

Since the off-flavors are believed to be due to oxidation the factors affecting the oxidation of butterfat have received increasing attention. In 1931 Briggs (27) showed that certain metallic catalysts exerted a great influence

in hastening the reaction. Ultra violet light, hydrogen peroxide, and fat peroxides had a strong pro-oxidative effect and lactic acid had a similar but less pronounced effect. The presence of curd exerted an anti-oxygenic effect, but glycerol, triolein, lactose, iodine, potassium iodide, high humidity and pasteurization had little or no influence. Henderson and Roadhouse (154) have shown that direct sunlight increases the percentages of unsaturated fats in the milk fat.

The form which copper takes in milk and milk products has been the object of different investigations. Osborne and Leavenworth (266) and Vandevelde (381) have shown that copper combines with the proteins. This combination seems to be a matter of adsorption rather than direct chemical combination. Most investigators believe that the copper ion acts in some way to catalyze the reaction whereby an oxidized flavor is produced. Smythe and Schmidt (334) have reported data which suggest that those substances which possess a particular grouping within the molecule will hold iron as an undissociated compound. Smythe (333) has presented a theory for the mechanism of iron catalysts. Warburg (385) and Goard and Rideal (124) believe that in oxidations by ferrous salts, the ferrous iron forms a peroxide and that this peroxide is the oxidant. Davies (84) has shown that iron and copper distribute themselves between cream and separated milk in proportion to the curd nitrogen, but upon complete centrifugation of the fat a higher concentration of metals occurs in the cream, indicating adsorption of complex proteinates at the fat-globule surface. The exact mechanism whereby either copper or iron catalyzes the off-flavor produced in milk and milk products is not as yet adequately explained.

As already mentioned Golding and Feilman (125) were probably the first to report the effect of copper on the flavor of milk. Since that time the effect of copper and iron on milk flavor have been noted by a large number of investigators (30, 45, 61, 63, 83, 88, 122, 126, 133, 141, 198, 212, 232, 245, 246, 276, 293, 300, 337, 342, 391). This flavor has been variously characterized as cappy, cardboard, oxidized, papery, metallic, metallic and oily, emery, oleagenous, and tallowy. It is generally agreed that this flavor is the result of oxidation and the term "oxidized" is now generally used.

Because of the importance of copper in milk Ritter (292) developed a test for copper based upon the peroxide reaction. Turgeon, Stebnitz and Sommer (379) modified this test slightly and found (380) that vitamin C complicated its application. Herrington and Brereton (158) developed a flame test for determining the presence of copper in a metal or alloy.

Sommer (335) has reported that fishy flavor in butter is the result of the production of trimethylamine from the lecithin. High acid, high salt, over-working, and the presence of iron or copper are factors favoring fishiness. Iron and copper salts act as oxidative catalysts. In comments following Sommer's paper, Hunziker states that he regards fishy and tallowy flavors

as being closely related in that both result from much the same combination of factors. Ritter and Christen (297) and Jensen and Ritter (193) also have shown that copper and iron contamination favor fishiness in butter fat but the former workers observed notable exceptions which indicated that other factors were involved. Horrall and Epple (176) have recently developed a modification of Ritter's test for copper in butter.

It has been known for some time that subsequent to the contamination of milk with copper, iron, or both, oxidized flavor may develop. The presence of these metals as contaminants does not always cause oxidized flavor, however, and the exceptions will be discussed in connection with the effects of feed of the cow and effects of processing (Sections VI and XI).

*Induction Period and Peroxide Value*—Considerable information has been gained by the use of the induction period method of Greenbank and Holm (137a), sometimes with minor variations. This method consists in maintaining fat at 95° C. in a gas-tight flask with agitation under an atmosphere of oxygen and measuring the amount of oxygen absorbed by the fat. Fats subjected to this treatment usually do not take up any considerable amounts of oxygen for some time. This period is called the induction period. At the end of the induction period rapid oxygen absorption begins. The length of the induction period is considered to be a measure of the resistance of a fat to oxidation. This method permits study of the effects of various added substances on the susceptibility of the fat to oxidation. Wright and Overman (409) found that the induction period of one butter fat was reduced from 32 hours to one hour when it was contaminated with copper. Briggs (27) found that the presence of certain metallic compounds shortens the induction period in decreasing order as follows: sodium vanadate, copper lactate, iron lactate, and nickel sulfate. He found that zinc lactate, on the other hand, increased the induction period slightly. Henderson and Roadhouse (154) added copper to milk, allowed it to stand for some time and found that the induction period of the fat of the milk had been reduced. A slight reduction was produced by nickel also, whereas 18-8 chromium-nickel steel produced no change.

The peroxide value of fats, as outlined by Wheeler (397), also has been used as a means of measuring the progress of oxidation. This method is based on the liberation of iodine from potassium iodide by peroxides formed in the fat. Since peroxides form during the oxidation of a fat, this value is considered to be an excellent indicator of the progress of the reaction. Ritter and Nussbaumer (301) found that copper added to butterfat accelerates peroxide formation in the presence of light. Later they reported (302) that small amounts of copper palmitate caused a large increase of peroxide value but large amounts caused little or no change. They found also that in butter which is tallowy already, increased amounts of copper inhibit peroxide formation, especially in the presence of heat and light.

Ritter and Nussbaumer (301, 302) have shown that the peroxide number of fat is a measure of its content of active oxygen, and therefore of its tendency to undergo oxidation. Their data show an increase in the peroxide number of a fat in the presence of light (especially in an unsaturated fat), air, copper, and a high temperature; a decrease in peroxide number occurs when 0.1 to 1.0 per cent of hydroquinone is added. Peroxide number was markedly reduced by heating butter fat at 195° and at 250° C., but such heating destroyed anti-oxidative properties, resulting in a marked increase in peroxide number upon later storage. Measuring the increase in peroxide number upon storage for 8 hours at 104° C. was an easy and reliable method of determining the keeping quality of butter. When air was passed through heated butterfat, the peroxide number increased rapidly. Copper in the form of fat-soluble copper palmitate, accelerates the assimilation of oxygen and increases accordingly the peroxide number of the fat, when it is stored in the dark at normal temperature. The higher the storing temperature, the more rapid was the increase of the peroxide number. The lower the peroxide number in this test the less the tendency for the fat to oxidize. When fat is being oxidized under the influence of heat there is a distinct increase in weight. Fats extracted according to the Roese-Gottlieb method very often show an extremely rapid increase in the peroxide number. Ritter and Nussbaumer demonstrated that the addition of increasing quantities of fat-soluble copper palmitate to butter fat caused the peroxide number to increase. The peroxide number increased more rapidly during storage of the fat in the dark at ordinary room temperature, as the concentration of copper palmitate increased within the chosen range of 0.000,001 to 0.33 per cent. On the other hand, when the copper containing fat was stored at ordinary room temperature in the light, it showed a slower increase in the peroxide number than fat which was free from copper. Later on the peroxide number of the butter fat contaminated with copper decreased in spite of a continued exposure to light. This was not the case with the copper-free fat.

On heating the samples during 8 hours at 104° C., fats which contained about 0.000,1 per cent of copper palmitate revealed the greatest increase, and fats with a greater copper content showed a lesser increase, of the peroxide number. The reason for this phenomenon is that copper not only accelerates the assimilation of oxygen to increase the peroxide number, but also hastens the further conversion of the fat peroxides. That is why the peroxide number decreases again in the presence of greater amounts of copper as well as in the presence of light and warmth. Moreover, a distinctly tallowy butter fat, when heated at 104° C. for 8 hours, gave a decreased peroxide number as the amount of copper palmitate added was increased. The nature of this second state of copper catalysis which brings about the destruction of the fat peroxides is not fully understood.

Wright and Overman (409) have shown an induction period of nearly 32 hours for fresh butter fat as compared with 5 hours for a 2 year old fat, 3

hours for butter fat containing 1 per cent lactic acid, and less than 1 hour for butter fats contaminated with copper. Temperature was found to accelerate oxidation of butter fat in proportion to the height of the temperature.

A great amount of work has been done on the various factors affecting rancidity (oxidative) in fats. Emery and Henley (97) have shown that air, oxygen alone or in combination, light, and metals are among the factors which influence the development of rancidity. Streaming CO<sub>2</sub> as a source of inert gas was found ineffective. Salkowski (318) regarded the development of rancidity in lard and butter fat as due to the combined activity of oxygen and light, and as a direct oxidation process in which the oxygen consumption could be measured. He believed that the speed of the reaction depended upon the intensity of the light and that little oxygen was consumed in the absence of light.

MacLean and Pearce (226) have shown that hydrogen peroxide with oleic acid in the presence of a copper catalyst brings about oxidation first at the 9th and 10th carbon atoms and then proceeds further in the 18-carbon chain. The oxygenated chain then breaks up. Succinic and oxalic were the only dibasic acids isolated. The 8 terminal carbon atoms of the oleic acid chain are broken off, and the remaining part of the chain appears to be completely broken up. The copper salt greatly increased the extent of oxidation. Clavel (49) studied the oxidation of oleins and concluded that there is a relationship between the drop in the iodine number of fats during storage and the adsorption of oxygen causing rancidity. Metals such as iron which are dissolved when fats are stored in metal tanks or containers catalyze the reaction.

The manner in which the oxidation of the fat takes place has been the subject for much speculation. Weiland and Franke (393, 394, 395) suggest that hydrogen peroxide can be used as the oxidant in studying the oxidation of organic acids in the presence of ferrous salts. Their conclusions are that the ferrous ion unites with the substrate thereby making the latter more susceptible to oxidation. On the other hand Warburg (385) and Goard and Rideal (124) believe that in oxidations by ferrous salts the ferrous ion forms a peroxide and that this peroxide is the oxidant.

Just what the mechanism is whereby the action of metals on the oxidation of fats is produced is difficult to demonstrate experimentally. However, the effect of metals on the quality of the product is well known and it is deemed advisable to keep the metal content as low as possible.

*Oxidation-Reduction Potentials*—One of the earliest works showing a relationship between oxidized flavor development and oxidation-reduction potential was made by Tracy, Ramsey, and Ruehe (366) in 1933. They showed that the normal tendency for freshly drawn milk was to shift toward a lower potential but that the addition of copper caused a shift toward higher potential. This was later verified by Thurston (355), Webb and Hileman (387)

and Greenbank (135). Thurston showed that iron, like copper, invariably raised the oxidation-reduction potential of the milk when an oxidized flavor was produced. His results showed that salts of tin and aluminum, when added to milk, produced a lower potential than that of normal milk and did not cause an oxidized flavor. Webb and Hileman (388) and Greenbank (136) showed that by the addition of copper and subsequent observation of the potential it was possible to predict, to a fair degree of accuracy, those samples which later become oxidized. Greenbank (135) has shown that a change from dry to green feed is paralleled by a decrease in oxidation-reduction potential and by an increase in the poising action (see Section VII "Effect of Feed"). It was his belief that the thermal inhibition of the flavor acted through a lowering of the oxidation-reduction potential. Webb and Hileman (387) have shown that summer milk does not develop oxidized flavors even in the presence of a high oxidation-reduction potential. Thornton and Hastings (354) and Fay and Aikins (102) found that the time curves of milk with and without methylene blue were in close agreement. Likewise, it was found by Kende (198) that milk which developed an oxidized flavor had a long reduction time as judged by methylene blue. Aikins and Fay (1) have shown that fat plays an important role in potential changes and that sunlight causes a drift toward lower potential. Likewise, light in the visible spectrum reduces methylene blue (189). Thornton and Hastings (354a) believe that reduction in milk is accompanied by the removal of oxygen by bacteria. Tracy and co-workers (366) have shown that incubation of milk prior to contamination with metal reduces the tendency for oxidized flavor to develop. Recently Strynadka and Thornton (344) have shown that abnormal udder conditions for milk with high leucocyte content were also responsible for abnormally high concentrations of reducing substances in milk.

Since it appears that anything which tends to lower the oxidation-reduction potential, tends to prevent the occurrence of oxidized flavor, it appears that many factors may indirectly or directly be involved in the development of oxidized flavor.

*Off-flavor in Ice Cream*—Tallowy or oxidized flavor in ice cream has attracted the attention of many different research workers (24, 69, 70, 73, 225, 314, 315, 365). Although tallowy flavor is generally associated with strawberry ice cream Ross (314) has pointed out that it may occur in any kind of ice cream but may be masked by the flavoring. The work of Dahle and Josephson (73) shows that vanilla ice cream will develop a tallowy flavor but that it will not acquire this flavor as readily as strawberry ice cream. Mack and Fellers (225) concluded the rapid flavor deterioration in strawberry ice cream was the result of oxidation of the butter fat. Working along this same line Ross (314) was unable to determine any changes in the degree of oxidation of fats of a magnitude that could be detected by the usual chemical

technique. The trend of the flavor development indicated that fat oxidation might be the cause of the defect. However, oxygen adsorption, as measured by the test for oxidase, gave no proof that oxidizing agents had any part in the flavor development. Iverson (188) states that the trend in iodine number of the fat points toward oxidation during the development of the off-flavor. Bird and co-workers (24) found a tendency for the drop in iodine numbers of the fats to be greatest in the samples which develop the defect most rapidly. Iverson (188) was unable to find any correlation between the initial and final Reichert-Meisel numbers and (the actual values were determined on the extracted fat at the beginning of the trial and after 60 days of storage) the development of the off-flavor.

Certain factors are related to the development of tallowy flavor in strawberry ice cream. Chief among these seems to be the presence of metallic salts (71, 188, 225, 365). Roundy and Jackson (315) observed that when condensed mixes were stored for 3 months and then made into ice cream, the lots made from mixes condensed in copper pans were criticized twice as often as those made in stainless steel or nickel pans. Ross (314) reports that iron does not seem to be a factor whereas Iverson (24, 188) found that samples high in iron showed a reduced tendency to develop an oxidized flavor. He suggested that possibly the iron was combined in the ferrous form and served as an anti-oxidant.

Mack and Fellers (225) reported that strawberries contain enzymes which aid in developing this off-flavor but Iverson and co-workers (24, 188) found that the oxidases introduced by the fruit are not the cause of the off-flavor. They found that if the berries were soaked in the mix before freezing the flavor development was retarded. Likewise, increasing the amount of fruit used has been found to retard the onset of tallowy flavor (71, 365, 367). Tracy, Ramsey and Ruehe (367) offer the explanation that the strawberries contain two agents affecting fat oxidation, one contained in the juice which serves as a catalyst while the other in the fiber serves as a reducing agent. Dahle and co-workers (69, 71) found that the off-flavor may occur as readily in pineapple ice cream as in strawberry and that the heating of berries and the ice cream mix to 180° F. for one hour did not prevent the development of this off-flavor. These results would lead one to believe that factors other than the oxidases in the fruit are responsible for the onset of this defect.

In their pioneer work Mack and Fellers (225) reported that the acid nature of the strawberries induced off-flavor development. This observation was borne out by the work of Dahle and Folkers (71) who reported the neutralization of the acid in strawberries delayed the onset of tallowy flavor. However, Tracy, Ramsey and Ruehe (367) reported that an increased citric acid content of the berries did not hasten or delay the onset of the flavor.

Dahle and Folkers reported in 1932 that no "cardboard" flavor developed in ice cream where dry skim milks were used in mixing formulas but

that it did occur in most samples containing condensed skim milk. This observation was later confirmed by Ross (314). He observed that samples made with condensed skim milk contained more copper and developed the cardboard flavor before similar samples made with dried skim milk. These results might be expected because larger amounts of solids were introduced by the condensed milk which probably contained more copper than the dried skim milk.

Dahle and Folkers (71) reported that the use of cardboard flavored milk or skim milk had little effect on the flavor of the ice cream but that sufficient copper was introduced through the condensed milk to cause the flavor. They observed that when the pans were kept well polished no off-flavors resulted. Bird and co-workers (24) reported that the copper content of dry skim milk samples ranged from 0.20 to 1.08 p.p.m. and the condensed skim milk samples ranged from 0.24 to 2.12 p.p.m. of copper. However, their results showed that ice cream made with dry skim milk as a source of serum solids developed oxidized flavors to a greater extent than those containing condensed skim milk. Ice creams made from condensed skim milk containing 2.7 p.p.m. of copper often developed oxidized flavor while on the other hand similar samples prepared from skim milk condensed in stainless steel pans did not show oxidized flavors. In the range between 1.18 and 1.8 p.p.m. of copper no predictions could be made as to the development of oxidized flavor defects. Apparently the state of the copper is more important than the total amount of copper present.

*Flavor Defects in Condensed Milk*—There is ample evidence to show that copper is detrimental to the flavor of condensed milk. Hunziker (177) and Rice (288) have pointed out that the manufacture of sweetened condensed milk in vacuum pans that have a coating of copper oxide on them results in a metallic- or tallowy-flavored product. The results of Rice indicate that the reaction is of a chemical nature and that the presence of air or oxygen greatly accelerates the rate of flavor development. Since condensed skim milk is much lower in fat content and much lower in flavor development than condensed whole milk the conclusion was reached that the flavor was the result of oxidation of the fat. Rice was able to produce tallowy flavor by adding copper and iron tartrate. Copper produced a much stronger flavor than iron and on this basis iron was believed to be of minor importance in the production of tallowy flavors in condensed milk. Both Genin (120) and Donauer (91) have observed the effect of copper on the flavor of condensed milk. Genin concluded on the basis of corrosion tests that practically all metals other than copper constitute better materials for the construction of evaporating pans. A factor which operates automatically to check the corrosion of metals in contact with the milk product being processed is the formation of a protective milk film. This protective film is less effective in the case of copper. Roundy and Jackson (315) observed that

although copper dissolved in the use of copper vacuum pans nevertheless it did not affect either the flavor or the keeping quality of evaporated milk. On the other hand Sommer and Gebhardt (337) found the flavor and color of evaporated milk impaired in direct proportion to the amount of copper present. They found appreciable amounts of copper dissolved in the making of Swiss cheese but reported no flavor defects due to the increased copper in this product.

#### IV. EFFECT OF AIR AND OXYGEN

*Butter and Butter Fat*—In 1890 Ritsert (305) reported rancidification as an oxidative change which required only the presence of oxygen and which could be accelerated by light. Later work (38, 192) showed that in addition to air, light and warmth greatly accelerated the rate of the reaction. As the result of his studies Jensen (194) came to the conclusion that air was important in butter deterioration only when accompanied by sunlight and high temperature. These results (192) indicate that olein is the point of attack when oxidation takes place.

Barnicoat (14) showed that practically no oxidation of butter fat occurred at 80° F. in an air pressure of 0.04 inches of mercury. Some inhibition of oxidation was noted in proportion to the extent of the vacuum used, at pressures of 3 to 10 inches, as compared to normal air pressure. Butter fat packed in tins under reduced air pressure was preferred to butter packed in and stored at atmospheric pressure for 3 to 6 months at 14° F. Flavor defects, when present, were mainly at the surface of the butter.

Hanus (152) observed that under the influence of air and light the saponification equivalent and the acid number of butter fat increased, whereas the iodine number decreased, and the Reichert-Meissl number remained unchanged. Butter subjected to the action of air and light gradually lost its yellow color, became lardy in appearance, smelled very rancid and had a sharp, tallowy taste. Nestrelayev (258) observed that butters from different parts of the country differed in their susceptibility to the action of light and air. The larger the content of unsaturated acid present the greater was the effect of light and air. The greatest changes produced by the action of light and air were in the Koeststorfer number, the average molecular weight of the non-volatile acids, and in the iodine adsorption number.

Browne (39) studied the spontaneous decomposition of butter fat over a period of 28 years by means of loosely stoppered samples and found a marked increase in the content of free and volatile acids and a considerable decrease in the content of insoluble acids. He advanced the theory that the disintegration of the fats was primarily due to the action of active or nascent oxygen, one atom of which was liberated for each atom absorbed at the points of unsaturation. Greenbank (134) obtained a negative Kreis test on butter fat following the removal of the free fatty acids by means of steam distillation, stored under vacuum in diffused light for three years. His study of the

effect of heat and air upon the oxidation of fats in the absence of light showed a fall in the induction period after 3 months from 248 minutes to 41 minutes.

Gray (131) observed that butter packed in full cans kept better than butter packed in partially filled cans. He attributed this difference to the action of air. In a discussion arising over the benefits to be derived from carbonation of butter Hunziker (179) reported that if butter is properly made it will have equally as good keeping qualities whether or not it is carbonated. Emery and Henley (97) found streaming CO<sub>2</sub>, as a source of inert gas, ineffective in preventing rancidity. However, Prucha, Brannon, and Ruehe (280) reported that the storage of butter in an atmosphere of CO<sub>2</sub> in tightly-sealed cans improved its keeping qualities, in that storage flavors did not develop as soon as in air and that mold growth was prevented. Since oxygen is necessary for the reaction whereby rancid and tallowy flavors are produced it seems reasonable to believe that the exclusion of oxygen would reduce or eliminate the development of these flavors.

Salkowski (318) in studying the rancidity of lard and butter fat believed it to be the result of the combined activity of oxygen and light, and that it was a direct oxidation process in which the oxygen consumption could be measured. Siegfield (329) studied the oxidation of butter fat and found that fresh fat exposed to light and air for 3 months increased in weight about 1 per cent and that the acidity increased nearly four fold. In addition he found increased Reichert-Meissl, saponification, and Polenske numbers, and a decrease in the iodine number. Holm (165, 167) studied the amount of gas taken up in the oxidation of fresh butter fat and found that an induction period was necessary before the oxygen was absorbed. Following the induction period oxygen was absorbed in a logarithmic manner. Determinations of acidity, iodine number, iodine liberated from potassium iodide in 24 hours, and Kreis tests were made. Of these determinations only the Kreis test gave results which would detect small differences in the degree of oxidation. He found no relation between oxygen absorbed and olfactory detection. A mixture of heptylic aldehyde and pelargonic acid added to fresh butter fat gave a tallowy taste but no Kries test.

Rogers *et al.* (307) used an especially designed apparatus and found that the volume of gas in freshly made butter was approximately 10 per cent by volume. Of this, 33 per cent was nitrogen, 20 per cent was oxygen and the remainder was absorbable by sodium hydroxide. They found the oxygen content was materially reduced after storage.

Aspegren (12) studied atmospheric oxidation and found that during exposure at 145° F. the nitrogen increased and the iodine number decreased.

Sommer (335) found that air was related to fishiness in butter. Overworking of butter tended to increase the air content of the butter and thus to increase the oxygen available for the oxidative production of trimethylamine.

*Dried and Fluid Milk*—Holm (165) found that tallowiness appeared first in samples of dry milk with the lowest moisture content. The dry product

of approximately 2 per cent moisture and the spray product of approximately 3 per cent moisture were found to be optimum for delaying tallowy flavors and odors. Samples above 4 per cent in moisture content always developed a fishy flavor. Whether or not the butter fat would absorb oxygen rapidly was found to depend not so much upon the quantity of oxygen available as upon the susceptibility of the fat. He pointed out that very small amounts of oxygen are necessary for the progress of oxidation. Somewhat contrary to what might be expected Holm *et al.* (171) found that in a five-month storage period air storage was best, vacuum storage was second and carbon dioxide storage was the poorest of the three methods, as judged by the induction period. These workers concluded that the inherent keeping quality of the fat was more important than the nature of the atmosphere in which it was stored.

Dahle and Palmer (76) found that milk powders high in moisture rapidly became tallowy when stored in moist air. They emphasized the point that containers must be absolutely tight to outside air and moisture to insure good keeping qualities. Palmer and Dahle (271) found that practically all the granules in spray process dried milk contained air cells and that the onset of tallowiness was found to be proportional to the size of the air cell. Coutts (59) also reported drum process powders superior to spray process in keeping quality.

Greenbank (133) has reported that aeration of milk increases the copper-tolerance of the milk. He found that aeration plus pasteurization gives the greatest protection against oxidized flavors. This does not appear to agree with the findings of Dahle and Palmer (78) who found that they could inhibit the development of oxidized flavor by the removal of oxygen. However, Brown, Tracy and Prucha (37) found that cappy or tallowy flavors usually developed more rapidly in vacuum capped milk. (This vacuum was insufficient to remove the dissolved oxygen). Hand *et al.* (143, 151) showed that the development of oxidized flavor could be largely or completely prevented by a process of vacuum cooling whereby the dissolved oxygen was largely removed from the milk. From these results it would appear that dissolved oxygen plays an important role in the development of oxidized flavor in milk. However, the amount of oxygen required to produce the off-flavor is very small and in order to prevent its development the oxygen apparently must be almost completely removed.

Tracy (362) reported a surface taint developed on frozen whipped cream in the hardening room. He described these odors as being absorbed from the air in the hardening room.

Although no work has been reported on the effect of air or oxygen on the development of oxidized flavor or tallowy flavor in ice cream, nevertheless oxygen might be expected to play a similar role in this product. The nature of the product is such as to insure an adequate amount of oxygen present for

the development of these off-flavors which would be expected to appear whenever other conditions were suitable. Here lower temperature evidently plays a role and aids in slowing down the rate of development of oxidized flavor.

#### V. EFFECT OF LIGHT

*Butter and Butter Fat*—In 1894 Von Klecki (383) observed that butter must be kept away from direct sunlight and at a low temperature to maintain its quality. About five years later Hanus (152) noted that butter subjected to the action of air and light gradually lost its yellow color, smelled very rancid, and had a tallowy taste. This was accompanied by an increase in acid number, and a decrease in iodine number. Nestrelayev (258) found that butters from different parts of the country differed in their susceptibility to change with exposure to air and light depending upon the content of unsaturated acids. Reinmann (287) expressed the opinion that light was not as important in causing rancidity as claimed by some investigators. Since that time various workers have observed the effect of light (27, 29, 38, 97, 103, 134, 154, 192, 202, 215, 218, 301, 302, 318, 329) and it has been established that light does have an important accelerating effect on fat oxidation. Ruemele (316) pointed out that it is the action of light and the composition of the fatty acid mixtures which determine the speed of fat spoilage. Stebnitz and Sommer (338) have shown that butter oil will oxidize by high temperature aeration in the absence of light, to produce a tallowy flavor in 3 hours. By use of ultra-violet light a tallowy flavor was produced in 15 minutes. The flavor produced by light differed somewhat from that produced by aeration alone. Sunlight likewise produced a tallowy flavor in 15 minutes, while diffused sunlight required 12½ hours to produce a tallowy flavor. Lamp light (100-watt at 10 cm.) gave a tallowy flavor after 4 days, whereas infra-red light did not cause a tallowy flavor in 14 hours, at which time the experiment was discontinued due to rise in temperature of the fat.

These findings have focused attention on the types of wrapping material used as a means of light control (17, 324). Davies (85) found that as a general rule the depth of color was more important than the actual color, in preventing oxidation. He observed that light blue was the least effective in preventing oxidation. This finding was verified by Morgan (248). Greenbank and Holm (138) in studying the relative accelerating effect of light of different parts of the visible spectrum on autoxidation of cottonseed oil, found the greatest acceleration in the range of the orange band. Holm, Greenbank, and Deysher (170) found that ultra-violet light had a powerful effect on the susceptibility of fat to autoxidation as judged by the induction period. Stutz, Nelson and Schmutz (345), reported that the effective region of light was limited to blue and the ultra-violet range as judged by the production of hydrogen peroxide. Coe (50, 51, 52), and Coe and LeClerc (53, 54, 55) found that selective ultra-violet light hastens the development of

rancidity as compared with natural sunlight. They found black and green containers a practical and effective means of protecting certain food materials against rancidity. Barnicoat (16) recommended the use of dim electric lights in packing rooms and the use of heavy grade parchment wrappers as a means of controlling light defects in butter.

Zilva (411) found that butter could be entirely bleached by exposure to ultra-violet light. In this process it became tallowy and lost its vitamin A value. Baumann and Steenbock (19) working with carrots and tomatoes found that pure carotene and vitamin A were destroyed by exposure to ultra-violet light but that crude carotene was entirely stable under the conditions of the experiment. Baumann and Steenbock (20) found that the maximum absorption of light in butter oil occurs at 460 and 485 mu. This is the point in the spectrum where carotene is determined by light absorption. Apparently carotene plays an important role in the effect of light on the autoxidation of butter fat, since butter is bleached in color prior to active oxidation as judged by the induction period.

*Milk and Cream*—The effect of light on milk was brought to the attention of investigators as early as 1907 by Burr (43). He reported that the exposure of milk in glass bottles to direct sunlight hastened its deterioration. He did not state the nature of the defect involved, but suggested the use of green, red, or black bottles. He also suggested the use of colored paper over plain bottles to hold back the injurious blue and violet rays. In 1930 Hammer and Cordes (150) reported that a tallowy flavor developed in milk following its exposure to sunlight in plain glass bottles, and that the development of this flavor was hastened by the presence of copper or iron known to be in the milk. Skimmilk developed a burnt flavor upon exposure but this flavor was not related to metallic salts and was not characterized as "tallowy." The exposure of milk in plain glass bottles to diffused light caused only a tallowy flavor, whereas the exposure of milk in brown glass to direct sunlight was without effect on flavor.

These early reports have focused the attention of investigators on the effect of light on milk. More recently the effect of sunlight on the flavor of milk has been noted by several workers (45, 107, 231, 232, 233, 276, 356). Marquardt (231) reports a bleaching effect from the exposure of milk to sunlight for 2 or more hours and an oxidized flavor after 20 to 60 minutes exposure followed by 24 hours storage. Greenbank (135) reports that light may inhibit, promote, or have no effect on the development of oxidized flavor, the results depending upon the contamination and the intensity of irradiation. Whitehead (400) studying the reduction of methylene blue found that methylene blue added to good quality milk reduced in a short time in the presence of sunlight but in darkness no decoloration resulted in 7 hours. This reaction was not due to an enzyme since it proceeded equally well in milk that had been heated at 100° C. for 30 minutes. The reduction of

methylene blue does not take place in skim milk (1, 44, 400) and for this reason it was believed related to the fat. Sodium oleate will restore the reaction while sodium palmitate does not restore it. It was suggested that sunlight catalyses the oxidation-reduction reaction in which unsaturated fats are oxidized and the methylene blue is reduced. Aikins and Fay (1), Jackson (189) and Buruiana (44) have observed the reduction of methylene blue by light. In addition to this Aikins and Fay observed that artificial light produced the same effect except to a lesser degree. These latter findings did not verify the results of Whitehead (401) who was unable to reproduce the effect of sunlight by use of either ultra-violet radiation or radiation from electric lamps. Davies (82) reports that exposure of milk to the radiation of a mercury-vapor lamp for 2 minutes was sufficient to change the flavor and shorten the induction period. This shortening of the induction period by exposure to ultra-violet light was verified by Anderson and Triebold (2). Genin (121) reported no loss of vitamin A from ultra-violet irradiation of condensed milks. Flake, Weckel and Jackson (106) found that it was possible to remove the activated flavor produced by irradiation by the addition of 2 or 3 p.p.m. of copper followed by bubbling air through the milk at 140-145° F. In addition, the odor of irradiated milk could be duplicated by irradiation of an aqueous solution of egg white or gelatin. This indicated that the protein fraction of milk was probably the parent substance of the flavor and that it probably was identical with the burnt flavor found by Hammer and Cordes (150). Because of the similarity between the burnt or activated flavor produced by sunlight and the tallowy or oxidized flavor which likewise is catalyzed by sunlight and the action of metals, considerable confusion has occurred in the literature due to terminology. Weckel and co-workers (390) found that it was feasible to irradiate the separated fat portions of the milk and to reconstitute the milk. In this way the reconstituted milk was relatively free from activated flavors.

Kon and Watson (208) have reported that light oxidized the ascorbic acid in milk. Their results show that light of short wave lengths is mainly responsible for this action. Ultra-violet light showed some activity while red and yellow lights were almost without effect. The replacing of the dissolved oxygen with an inert gas prevented the action of light. More recently Kon (207) reports that under the action of light the ascorbic acid of milk undergoes reversible oxidation, most probably to dehydroascorbic acid. He reports that the power of ascorbic acid to reduce indophenol, lost through exposure to light, can be restored to an extent varying with the length of exposure by treating the milk with hydrogen sulfide. Hopkins' (175) work gives a clue as to the mechanism by which light destroys the vitamin C in milk. In the presence of light, lactoflavin rapidly causes the destruction of vitamin C, there being a simultaneous degradation of lactoflavin to lumichrome. The work of Hand, Guthrie and Sharp (151) verifies this mecha-

nism. They observed that after the lactoflavin and ascorbic acid are destroyed by prolonged exposure to sunlight, any additional ascorbic acid added to the milk is relatively stable. The sensitivity of ascorbic acid to light can be restored by the addition of lactoflavin to the milk.

Guthrie, Hand and Sharp (143) report a general relationship between the factors which accelerate the rate of oxidation of ascorbic acid and the production of oxidized flavor. The effect of sunlight in causing the oxidation of ascorbic acid is due to the photosensitizing action of the lactoflavin and the presence of oxygen in the milk. Paper bottles markedly decrease the effect of sunlight on the oxidation of ascorbic acid and on the production of off-flavors.

Doan and Myers (90) studied the effect of sunlight on some milk and cream products. Paper bottles, of the type used, offered appreciable protection to skim milk, whole milk and butter milk against the action of sunlight in producing burnt flavors as compared to the use of clear glass bottles. The paper bottles were of no protection to whole milk, cream, or homogenized whole milk against tallowy flavors caused by sunlight. Blue and green colored paper bottles or blue and green cellophane wrappers on glass bottles retarded the development of tallowiness and burnt flavors. They found that the degree of tallowy flavor produced in milk and cream by sunlight was stronger in the paper bottles than in the clear glass bottles.

Apparently light plays a role in the development of oxidized flavor in milk and cream both by its effect on the carotene content and by its indirect effect through lactoflavin and vitamin C.

#### VI. EFFECT OF FEED GIVEN THE COW

*Composition of Fat*—One of the early investigations of the effect of feed on butter fat was made by Hunziker, Mills and Spitzer (184) in 1912. Their work showed that feeding cows rations high in linseed oil meal or cottonseed meal will increase the iodine number of the fat. Henderson and Roadhouse (154) found that when animals are on submaintenance rations they draw upon their body fat for milk production and thereby increase the percentage of unsaturated fats in the milk and increase the susceptibility of the fat to oxidation. Hening and Dahlberg (156) found that feeding cows at a level below the Morrison standard did not influence either the flavor of the milk or the percentage occurrence of oxidized flavor in the milk. Hill and Palmer (160) found that when barley constituted from 35 to 50 per cent of a low-fat diet containing alfalfa or timothy hay a hard butter fat with a low iodine value was produced. Dahle and Carson (69) report that cows fed alfalfa hay produced milk more susceptible to oxidized flavor than cows on other roughages. Hill and Palmer reported that the inclusion of oils or fats in the ration resulted in butter fat which assumed some of the characteristics of the oil fed. Linseed oil in the ration significantly increased the content of fatty

acids less saturated than oleic acid. Prewitt and Parfitt (278) found that rations containing soybean oil, either as such or in the unprocessed beans, had a tendency to produce milk which upon holding developed less intense oxidized flavor than did milk from cows fed other rations. Dean and Hilditch (86) found that when cows went on grass there was an abrupt increase in the oleic and linoleic acids and a parallel reduction of butyric and stearic acids in the butter fat.

*Green and Dry Feed*—One of the earliest workers to report a seasonal variation in oxidized flavor was Mattick (237) who in 1927 reported that oiliness in milk appeared in autumn, winter and spring, but never in the summer. Kende (198) recognized this fact and as the result of his work concluded that green feed contained some substance or substances which when fed to the cow protected the milk against the off-flavor. Since that time numerous workers (31, 40, 64, 135, 142, 276, 279, 355, 364, 372) have observed the difference in susceptibility of winter and summer milk to oxidized flavor. Stebnitz and Sommer (340) found that when cows received grass as part of their ration, the butter fat became less saturated and more susceptible to oxidation. However, it appeared that protective substances in the milk prevented the development of oxidized flavor. Green feeds (13, 31, 64, 78, 198, 355, 356) have been shown to yield a more stable milk as compared to milk produced on dry feed. Majer (227) has shown that the following action of beet forage can be prevented by the addition to the ration of fresh Alp hay. In contrast to this, Henning and Dahlberg (157) reported that the feeding of mangels or beet pulp in no way prevented or increased the susceptibility of milk to oxidized flavor development.

*Vitamin C and Carotene*—The nature of the inhibiting substances carried in green feed has been the object of extensive investigations. The first clues as to the probable nature of these substances were found by Ritter (291) and Chilson (47) in 1935 when they found that the addition of ascorbic acid to the milk prevented the development of oxidized flavor. These findings were verified by Sharp, Trout, and Guthrie (328) and by Dahle (64, 78). Sharp *et al.* found a positive correlation between the rate of oxidation of ascorbic acid and the rate of development of oxidized flavor. Trout and Gjessing (374) found the percentage decrease of ascorbic acid upon storage greater in the winter than in the spring, summer or fall. Brown, Thurston and Dustman (31) found that the feeding of one quart per animal per day of either tomato or lemon juice to cows on dry feed reduced the susceptibility of milks to oxidized flavor development. They attributed this effect to the ascorbic acid in the feed and observed a similar tendency when pure crystalline ascorbic acid was fed at the rate of  $\frac{1}{2}$  gram daily. Riddell *et al.* (290) found 25.8 mg. of ascorbic acid per liter of milk produced by cows on a dry ration plus silage, 26.5 mg. per liter by cows on dry ration alone, and 26.5 mg. per liter by cows on pasture alone. Rasmus-

sen and others (285) found that the stage of lactation appeared to have a more definite effect upon the ascorbic acid content of the milk than did breed differences. The ascorbic acid content of milk was found to be relatively high during the early stages of lactation, but decreased to a minimum after about two months of lactation and then increased to a maximum in the later stages of lactation. Brueckner and Guthrie (40) found no relation between the period of lactation and the development of oxidized flavors. Whitnah, Martin and Beck (403) ranked the breeds in descending order according to the average vitamin C content of the milk as Jersey, Guernsey, Ayrshire, and Holstein. The frequency with which spontaneous oxidized flavor occurred in their study was in the reverse order. Garrett, Tucker, and Button (115) found that the average flavor scores show that for each ascorbic acid class interval where a decrease of acid occurs, a decrease in flavor score also occurs. They found the apparent critical point of relationship of ascorbic acid and good flavor to lie between 15 and 18 mg. of ascorbic acid per liter of milk. Beck, Whitnah and Martin (22), however, found no relation between the amount of vitamin C in the original milk or the amount of vitamin C lost during storage and the development of oxidized flavor in milk. These findings appear to be somewhat at variance with the findings of Sharp, Trout and Guthrie (328). Dahle (64) likewise found that when oxidized flavor occurs naturally in milk the ascorbic acid is greatly reduced.

Garrett, Tucker and Button (115) found a close relationship between percentage of fat and color, between color and the first day ascorbic acid content, and between color and first day flavor. It was their belief that both carotene and ascorbic acid had reducing properties and that they acted in this way to exert protective action on the milk.

Garrett, Arnold and Hartman (112) reported that the feeding of grass silage had a greater stabilizing effect on ascorbic acid in milk than corn silage or beet pulp. This stabilizing effect tended toward milk of better flavor.

Whitnah, Martin and Beck (403) found that within the breed there was no relation between the amount of vitamin C present and the development of oxidized flavors from individual cows. All samples which developed oxidized flavor were below the breed average in intensity of fat color. However, they found samples low in color which did not develop oxidized flavor. They were able to reduce oxidized flavor by feeding 2 pounds of dehydrated oats (young plants) containing 103 mg. of carotene per pound. The flavor defect was completely eliminated by feeding a carotene concentrate containing 150 mg. of carotene per pound.

Anderson (6, 7, 8) and co-workers (10) were some of the earliest workers to show the effect of carotene on the flavor of milk. Their work showed that carrots or machine-cured alfalfa fed to cows would eliminate oxidized flavor in their milk. This they correlated with the carotene content of the feed.

Anderson, Hardenbergh and Wilson (9) obtained far more effective results in the elimination of oxidized flavor from the feeding of 8 pounds of carrots in the daily ration than was obtained from the addition of 500,000 U. S. P. units of vitamin A. Whitnah, Peterson, Atkeson, and Cave (404, 405) and Beck, Whitnah and Martin (22) report that a carotene supplement quickly corrected the tendency for an oxidized flavor to develop spontaneously. Brown, VanLandingham and Weakley (35) found that a carotene supplement added to the ration rendered the milk less susceptible to metal-induced oxidized flavor. Likewise, it was their finding that supplementing a low carotene ration with ascorbic acid produced similar results. Martin (233) reports that to minimize the occurrence of milk with oxidized flavor the milk should be kept normal with respect to carotene content. Dahle (67) was unable to delay the onset of oxidized flavor by the direct addition of carotene to the butter fat.

Booth *et al.* (25) found a close agreement between the amount of green material in the diet of the cow and the carotene and vitamin A content of the butter fat. It was concluded that the vitamin A activity of the summer butter fat appeared to be three times greater than that of winter fat, and that the fraction of total activity due to carotene was also greater in summer butter fat. Hilton, Hauge, and Wilbur (161) found that under winter feeding conditions timothy hay produced a butter low in vitamin A value, while good quality alfalfa or soybean hay were effective either in maintaining the high vitamin level of butter or in restoring its summer value. They found that the vitamin A value of butter responded rapidly to changes in the ingestion of vitamin A by the cows. Baumann and co-workers (21) found variations in vitamin A content of as much as 100 per cent between individuals of the same breed. They found that 3.3 per cent of the vitamin A ingested on a low carotene ration was recovered in the milk, but that only 1.3 per cent was recovered from a high carotene ration. Loy *et al.* (222) found that 11 days were required to reach an equilibrium on a carotene depletion ration and that during the repletion period the rise was quite rapid and reached an equilibrium level in about 10 days. Tucker, Garrett and Bender (378) found a close correlation between high yellow color and good flavor in milk. They reported that grass silage was superior to either corn silage or beet pulp in the production of good flavored milk. In a later work (113) they suggest the use of grass silage as a method of rendering milk less susceptible to oxidized flavor. Hodgson, Knott and Murer (163) found that hays were a poor source of vitamin A while silages and grass were the best sources. The carotene content of the butter fat accounted for only 11.2 to 12.7 per cent of the total vitamin A activity. Henderson (153) in a review of literature points out the desirability of having winter rations high in carotene content.

Thurston (356, 357) reviewed the literature on oxidized flavor and suggested the following classification for milks from the standpoint of oxidized flavors.

1. *Spontaneous milk*—Milk capable of developing oxidized flavor spontaneously, i.e., without the presence of iron or copper as a contaminant.
2. *Susceptible milk*—Milk which does not develop oxidized flavor spontaneously, but is susceptible if copper or iron contamination occurs.
3. *Non-susceptible milk*—Milk which will not become oxidized even when contaminated with iron or copper.

Since both vitamin A and carotene have been reported to have antioxidant properties (see section XIV) any factors which affect these substances may have a relation to oxidized flavor. In 1920 Hopkins (174) reported a rapid destruction of vitamin A by heat and aeration. Matill (234) found that the oxidative changes which accompany the beginning and development of rancidity in unsaturated animal fats tend to destroy vitamins A and E. This process is hastened by ferrous iron. Simmonds *et al.* (330) observed the so-called salt ophthalmia in animals fed rations containing ferrous sulphate. A cure was effected when rations were made up daily. Jones (195) observed the same effect and believed it due to the iron catalyzing the oxidative destruction of vitamin A. McCollum *et al.* (239) confirmed this finding. Marcus (229) believes that the destruction of vitamin A by granulated lactose is an autoxidation in which hydroquinone prolongs the induction period. Taylor (351) reported both vitamin A and E destroyed by ferrous chloride.

Hilton, Hauge and Wilbur (161) found good quality alfalfa or soybean hay were effective either in maintaining a high vitamin A level in butter or in restoring it to its summer value. The vitamin A value in butter responded rapidly to changes in the ingestion of vitamin A by the cows. Hodgson and co-workers (163) found home grown field cured hay the poorest source of vitamin A of the different roughages tested. Bartlett *et al.* (18) found that kale and artificially dried grass markedly increased the vitamin A content of the milk but that sprouted maize and mangels had little effect.

In general it appears that both ascorbic acid and carotene are related to the susceptibility of the milk to oxidized flavor. Ascorbic acid acts either from its presence in the feed or by being placed directly into the milk. From the results available it does not appear that vitamin C in the feed passes directly into the milk to prevent the flavor. However, it probably acts in some way as a stabilizer for the vitamin C secreted in the milk. Carotene apparently must be in the feed in order to effect the susceptibility of the milk. Both of these substances have reducing properties and it is possible that through these properties they may affect the susceptibility of the milk to oxidized flavor.

## VII. EFFECT OF ACID

*Cream and Butter*—In the early days of the butter industry, butter made from sour or ripened cream was believed to have superior keeping qualities to sweet cream butter. As early as 1890 Patrick (274) was studying the alleged poor keeping qualities of sweet cream butter. Later, Patrick, Leighton and Bisbee (275) found that sweet cream butter retained its flavor better than butter made from sour cream. In 1904 Michels (242) reported that butter of the best keeping quality was obtained from well ripened cream. He recognized, however, that if the ripening process was carried too far undesirable keeping qualities would result. McKay and Larsen (241) state that in the ripening process the lactic acid bacteria suppress the other types which if carried into the butter would produce undesirable changes. Wiley (407) observed less deterioration in butter made from cream acidified with lactic acid than in butter made from cream ripened to the same pH. McKay and Larsen recognized the danger of carrying the ripening process too far. Many research workers (27, 92, 147, 165, 220, 223, 267, 268, 384, 399) have noted the detrimental effect of ripening cream on the keeping quality of the butter. Mortensen (251) found that the per cent acid in cream was inversely proportional to the keeping quality. He also observed that proper neutralization gave good results. Flake (105) found that butter falling within the range of pH 6.0 to 6.49 had better keeping qualities than did butter of either higher or lower hydrogen ion concentration. Ellington (95) found that a serum pH of 7.0 or over indicated a low quality of butter due to over-neutralization or low quality raw material. Overman *et al.* (268) found no differences in keeping quality due to use of different neutralizers. However, over-neutralization resulted in low initial scores and low keeping quality. That pH has an effect on the rate of oxidation of fat, in general, has been shown by the work of Lea (216). He found the rate of oxidation of lards showed a sharp increase as the reaction became alkaline. Apparently this explains the effect of over-neutralization in butter.

Rogers and co-workers (308, 309) found that acidity was an important factor in the development of fishy flavor in stored butter. The work of Ritter (294) verified these findings. Ritter and Christen (299) found that certain alkali producing bacteria retarded the development of fishiness in butter. Haglund and Walker (148) found that the tendency to develop fishy flavor could be reduced by partial neutralization of the acid in ripened cream. In 1923 Sommer (335) found that fishy flavor in butter was the result of the chemical decomposition of lecithin with the production of trimethylamine as the immediate cause. The work of Jensen and Ritter (193) showed the development of fishy flavor to be brought about by hydrolysis and oxidation of the lecithin. At about the same time Holm, Wright, White, and Deysher (172) correlated the deterioration of butter in storage with a score below 89, with chemical changes. These changes were

brought about by oxidation. From these results it would appear that acidity plays an important role in the deterioration of butter which under some conditions appears to be the result of oxidation.

*Milk*—Anderson (3) and co-workers (5) found a relationship between apparent acidity and the susceptibility of milk to oxidized flavor. It is generally known that winter milk is higher in acidity than summer milk and that winter milk is more susceptible to oxidized flavor than is summer milk. They observed that the neutralization of high acid milk to 0.145 per cent acidity or lower was effective in the prevention of oxidized flavor. Likewise, they observed that high acid milk invariably developed an oxidized flavor after pasteurization. In a study of 220 individual samples of winter milk, Brown and Dustman (33, 34) were unable to find any correlation between the acidity of freshly drawn milk and its tendency to develop oxidized flavor when contaminated with copper. They were unable to prevent the development of oxidized flavor by neutralization to 0.13 per cent titratable acidity or in a small number of cases to as low as 0.10 per cent. These workers were unable to offer any explanation as to the disagreement of their results with those of Anderson and his co-workers other than the fact that Brown and Dustman added the copper from a solution of copper sulphate, whereas Anderson and co-workers depended upon contamination from the equipment as a source of copper. From a theoretical point of view it seems unlikely that natural acidity plays an important role in the development of oxidized flavor in milk when copper contamination takes place.

### VIII. EFFECT OF MOISTURE

*Butter and Butter Fat*—In 1915 Hyland and Lloyd (185a) found that the complete oxidation of fat was possible in dry air, whereas moist air prevented oxidation of glycerides and assisted oxidation of free fatty acids. Later Fierz-David (103) expressed the belief that rancidity was produced by air, light, and water without the action of bacteria; that unsaturated fatty acids were split into aldehydes and acids; and that fats containing saturated fatty acids were oxidized to the corresponding methyl alkyl ketones. Brochot (29) reported that for cold storage of butter the condensed water from the cold surfaces in the storage chamber should be eliminated as far as possible. He emphasized the importance of low-humidity in the butter cold storage chamber. Holm (165) using an oxygen absorption method found that the presence of water in a fat extended the induction period. Ritter and Nussbaumer (303) explained these results on the basis that butter serum must contain some substance or substances which retard the oxidation of the butter fat. They found that the rate of oxidation of pure butter fat was scarcely affected by the addition of a quantity of water equal to that contained in butter. From their results it would appear that in butter the moisture itself does not play as important a role as does the material which

it carries. Ritter and Nussbaumer explained this effect on the basis of the cephalin and lecithin content of the serum. Both of these substances exhibit antioxidative properties.

**Dried Milk**—Dahle and Palmer (76) reported that milk powders high in moisture and stored in moist air rapidly became tallowy. Davis (80) likewise, reported that for a milk powder to possess good keeping qualities it must be low in moisture. In contrast to these results, Holm, Wright and Greenbank (171) found that in general more rapid deterioration occurred in samples of low moisture content. In another study Holm (165) found that an increase in the moisture content of a milk powder not only retarded oxidation but prevented the formation of those intermediate products which gave rise to a tallowy odor.

#### IX. EFFECT OF SALT IN BUTTER

In 1903 Buhl (41) reported that light salting of butter had an advantage over heavy salting in the keeping quality of butter. Gray and McKay (131) likewise found that butter containing low percentages of salt kept better than butters containing high percentages of salt. Washburn and Dahlberg (386) reported that salt exclusive of its antiseptic property hastened deterioration of butter. Oily, metallic and fishy flavors showed up to a greater extent in salted than in unsalted butter. Guthrie, Scheib and Stark (146) reported that salt in butter greatly retards bacterial growth. Butters containing no salt were prone to become old or stale but seldom oily. These findings presumably can be attributed to inhibited bacterial growth since the storage was carried out at 10° C. Kildene (203) reported that salt accelerated the deterioration of butter at temperatures which check bacterial growth but at higher temperatures the salt inhibits the growth of the bacteria and thus preserves butter. The work of Overman and co-workers (267, 268) showed that salted sweet cream butter had a slightly higher initial score but lost score more rapidly than unsalted sweet cream butter.

The results for butter storage are not clear cut. Sayer *et al.* (322) used a storage temperature of about 5° F. and reported no differences in keeping quality for butter with salt contents from 1.06 to 3.78 per cent. Rann, Brown and Smith (284) reported that salted butter keeps better than unsalted butter above the freezing point as well as below. Jacobsen (191) reported that the destructive action of salt apparently was of greater importance than freezing in reducing the number of bacteria in salted butter. Flake (105) found little or no relation between salt concentration and keeping quality, except in the samples which contained less than 2 per cent salt. Here there was a slight decrease in keeping quality.

Since high salt concentration is associated with the development of fishy flavor and since fishy flavor apparently is the result of hydrolysis and oxidation of lecithin, it would seem that salt tends to favor oxidative changes.

## X. EFFECT OF STORAGE TEMPERATURE

In 1894 Von Klecki (383) reported that storage butter should be kept at a low temperature to prevent deterioration. Thomsen (353) reported that butter from good cream kept better than butter from poor quality cream. Barnicoat (15) found that freezing temperatures are superior to chilling temperatures for butter storage. Likewise, he (16) found that butter is more susceptible to color and flavor defects when held at relatively high temperatures. Rogers, Thompson and Kiethley (309) stored butter at 0, 10, and 20° F. Of these three temperatures the best results were obtained at 0° and the poorest at 20° F. Gray and McKay (131) stored butter at -10, 10 and 32° F. and found that butter kept best at -10° F. Holm and co-workers (172) reported that drop in storage temperature from -10 to -17° F. does not result in an increase in keeping quality proportionate to that observed by a similar lowering at a higher temperature range. Ellis (96) found that at 75° C. stearic acid shows appreciable autoxidation under certain conditions of dispersion and catalysis. Wright and Overman (409) found increase in temperature to accelerate oxidation of butter fat in proportion to the height of the temperature.

From these results it would appear that temperature has a direct bearing upon the rate of oxidation of butter. The higher the temperature the more rapid the rate of oxidation.

Dahle and Palmer (76) reported that "tallowy" and "stale" are the outstanding flavor defects in dry milk powders. They found that storage at 4 to 20° C. gave about equal results with sealed tin containers but that 37° C. was too high.

Seism (326) found that ice cream kept better at -25° F. as compared to retail cabinet temperature. The very nature of the product insures a low temperature during storage and a dipping temperature that is within a relatively narrow range.

When cream is stored, the exact temperature does not appear to be of great importance compared with metallic contamination, as shown by numerous investigations (94, 249, 250, 321, 342).

The effect of storage temperature on the susceptibility of milk to oxidized flavor has not been determined as such for the reason that it is obscured by the effect of bacterial growth at 50° F. or above. (See section II.)

## XI. EFFECT OF PROCESSING

*Milk, Cream, and Butter*—The effect of heat in the processing of cream has been the object of extensive investigation. White and Campbell (398) found that pasteurizing cream at temperatures between 145 and 165° F. did not improve the keeping quality of the butter as judged by the score. On the other hand, Guthrie, Scheib and Stark (146) found that pasteurization of cream at 165° F. produced butter of better keeping quality than pasteuriza-

tion at 145° F. They attributed this result to the destruction of oxidases at 165° F. whereas, they stated only lipase is destroyed at 145° F. Mortensen (251) reported slightly better quality in fresh butter from sour cream pasteurized at 170° F. for 20 minutes, than in butter pasteurized at 145° F. for 30 minutes. However, the butter pasteurized at the lower temperature kept better in storage. Pasteurization at 170° F. for 20 minutes was better than at 180° F. for 20 minutes. Crews (60) reported that pasteurization at 165° F. for 30 minutes destroyed most, if not all, of the harmful milk enzymes. He believed oxidation to be the most important factor in butter deterioration. Ritter (294, 295) found that a fishy flavor could be prevented by flash pasteurization of cream at 90° C. Greenbank and Holm (137) found that heating fat at 100° C. to destroy any enzymes reduced the keeping quality in proportion to the length of heating.

Enzymes have long been considered as a factor in the deterioration of butter. However, with present pasteurization methods the activity of the enzyme lipase has been largely eliminated. In 1904 Rogers (306) reported that deterioration of canned butter was due to enzymes from the milk or enzymes produced by micro-organisms. In 1913 Winckel (408) reported that enzymes and ferments had little, if any, effect on the deterioration of butter in storage. Palmer and Combs (270) found that tallowy flavor in butter resulted from the addition of copper lactate to either raw or pasteurized cream. A pasteurization temperature of 79–80° C. for 15 minutes was used. In a later work Palmer and Miller (272) found by the addition of peroxidase to butter that this enzyme was not a factor in the deterioration of butter. Dahle and Palmer (77) reported the presence of peroxidases in fresh milk powder. However, the factors known to favor oxidation proved detrimental to peroxidase activity. Thacher and Dahlberg (352) were able to show indications of oxidase action in milk by use of pyrogallol. Boiling the milk failed to completely inhibit this action. Proks and Groh (279) reported that lipolytic enzymes were responsible for the development of oily-rancid and tallowy flavors in milk. They found that the degree of off-flavor developed was proportional to the quantity of lipolytic enzyme present.

The belief that oxidized flavor is the result of the action of an enzyme has been based largely upon the fact that milk heated to very high temperatures does not develop the flavor. Kende (198) in 1932 reported that milk heated to 85° C. did not develop an oxidized flavor. He named the enzyme responsible for the flavor "oleinase." Majer (228) was unable to verify the findings of Kende although he did find that the high heat treatment greatly reduced the intensity of the flavor developed. Guthrie and Brueckner (142) reported that pasteurizing at 160° F. or higher for 30 minutes decreased or prevented the tendency for spontaneous oxidized flavor to develop. Dahle (64, 66) reported that heating milk to 145° F. for 30 minutes intensified the oxidized flavor developed but that heating to 170° or above eliminated the

flavor defect. Brown, Thurston and Dustman (30) found that copper added after pasteurization developed a more intense oxidized flavor than copper added prior to the pasteurization process. Gould and Sommer (127) were able to correlate this finding with the development of a cooked flavor in the milk.

The recent work of Gould and Sommer (127) and Gould (128, 129) has shown that sulphur compounds are liberated when a cooked flavor is produced. They were able to develop an oxidized flavor in milk heated to 90° C. These findings tend to disprove the action of an enzyme in the development of oxidized flavor in milk. In addition, the production of a cooked flavor liberates sulfides which act as antioxidants. These findings have been verified by the work of Josephson and Doan (196). Greenbank (135) reported that the thermal inhibition of oxidized flavor acts through a lowering of the oxidation-reduction potential. This has been confirmed by both the work of Gould and Sommer (127) and Josephson and Doan (196), who found that the sulfide compounds, liberated when a cooked flavor develops, lowered the potential.

Whitnah *et al.* (406) reported excessive losses of vitamin C from holding process pasteurization, even when copper contamination was reduced to a minimum. Sharp, Trout and Guthrie (328) reported that low-temperature pasteurization did not accelerate appreciably the destruction of ascorbic acid, provided contamination with copper was prevented. They found a positive correlation between the rate of oxidation of ascorbic acid and the rate of development of oxidized flavor. Sharp (327) believed the destruction of vitamin C in raw and pasteurized milk to be due to the action of an oxidizing enzyme which is destroyed at temperatures higher than pasteurizing temperature. Since both the development of oxidized flavor and the oxidation of ascorbic acid are prevented by high temperature pasteurization, it seems possible that the development of a cooked flavor with the production of reducing substances also may play a role in the oxidation of ascorbic acid.

Homogenization of milk was first reported to retard the development of oxidized flavor by Tracy, Ramsey and Ruehe (366) in 1933. Since that time Thurston, Brown and Dustman (360), Ross (313) and Dahle (67) have observed the deterrent effect of homogenization on the development of oxidized flavor. Trout and Gould (373) reported that homogenization did not retard the development of oxidized flavor when high rates of copper contamination occurred. This probably explains the tallowy flavor observed by Doan (89) in homogenized milk.

Thurston, Brown and Dustman (360) reported that prolonged agitation at a low temperature, and freezing followed by thawing reduced or eliminated the susceptibility of milk to oxidized flavor development. Their work showed that prolonged agitation of milk at low temperature caused a transfer of the lecithin from the fat globule surface to the plasma portion of the

milk. It was their belief that lecithin in the plasma portion would not become oxidized readily under normal conditions and hence the oxidized flavor failed to develop fully after these treatments.

Dahlberg and Carpenter (63) found that the development of oxidized flavor in pasteurized milk was accelerated by contact with a clean copper-alloy metal, especially following chlorine sterilization, but this effect became less as more milk continued to pass through the equipment. Tracy and Ruehe (368) reported that chlorine sterilizers added to milk containing copper hastens the development of tallowy flavor. Grant (130) found that certain chlorine sterilizers greatly accelerated the rate of corrosion.

*Ice Cream and Condensed Milk*—Oxidized or tallowy flavor has long been a serious defect in strawberry ice cream. Scism (326) and Schricker (325) reported that chocolate ice cream has superior keeping quality to vanilla and that strawberry has the poorest keeping quality of these three ice creams. Various attempts have been made to avoid the development of oxidized flavor by variations in the percentage of fruit used and by various treatments of the fruit. Dahle and Folkers (71) and Tracy *et al.* (365, 367) reported that increased amounts of berries used in the ice cream delayed the onset of tallowy flavor. Tracy and co-workers found that soaking the fruit in the mix prior to freezing also delayed the onset of the flavor. Likewise, it was their finding that heating the berries at 150, 175, 200° F. and autoclaving at 15 pounds pressure for 15 minutes, decreased progressively the flavor defect. However, they were unable to eliminate the flavor by this procedure. Likewise, Dahle and Folker (71) were unable to eliminate the flavor by heating the mix and berries at 180° F. for one hour. They reported that neutralizing the berries to pH of 7.0 delayed the appearance of the tallowy flavor but injured the flavor of the berries.

Mack and Fellers (225) reported that enzymes contained in the fruit may be a factor in the development of a tallowy flavor. Tracy, Ramsey and Ruehe (367) found that oranges, lemons, pineapples, apples, peaches, and apricots cause the flavor when used in 3 to 10 per cent quantities. When used in large (25 per cent) quantities these fruits like strawberries did not cause the flavor to develop. The enzyme oxidase has been found in practically all of these fruits (101, 286). Mudge and Tucker (254) reported that strawberries which had been aerated by drawing air through them for a considerable time produced a stale and unclean-flavored ice cream. Ross (314) studying oxygen absorption, as measured by the test for oxidase, found no proof that oxidizing agents had any part in the development of the off-flavor. Iverson (187, 188), and Bird and co-workers (24) reported that oxidases from the fruit do not cause the oxidized flavor to develop. These results, together with the finding that extreme heating of the berries does not eliminate the undesirable flavor, suggest that this defect is caused by factors other than the presence of an oxidative enzyme.

The inhibiting effect of homogenization on the development of stale, metallic flavors in ice cream has been noted by Dahle and coworkers (69, 73) and by Tracy, Ramsey and Ruehe (365, 367).

Tallowiness in condensed milk has been noted by various investigators (91, 141, 177, 288). Sommer and Gebhardt (337) reported that evaporated milk was found to be impaired in flavor in direct proportion to the amount of copper present. Holm *et al.* (169) found that as the fat content increased the keeping quality decreased. Homogenization and precondensing were found to improve the keeping quality. Recently Corbett and Tracy (57) reported that condensing milk under vacuum to a concentration of approximately twice the original solids content prevented the development of oxidized flavor in both the condensed milk and the condensed milk reconstituted to the original concentration. These workers used soluble copper at the rate of 3 p.p.m. for contamination. They found that the retarding effect of condensing was on the plasma portion of the milk and explained this phenomenon on the basis of liberation in the serum portion of the milk of certain antioxidative constituents that are probably derivatives of the milk proteins. This description seems to fit the sulphhydrils described by Gould and Sommer (127) and by Josephson and Doan (196). Ordinarily one would not expect evaporated milk to develop an oxidized flavor because of the high heat used in sterilization and the homogenization treatment which the milk receives.

## XII. COMPOUNDS FORMED AND THEORIES OF THEIR FORMATION

*Oxidation Products of Oils and Fats*—Scala (323) isolated the decomposition products of rancid fat, and by oxidation with potassium permanganate was able to obtain acids which were separated by means of barium salts.

These acids were identified as oenanthyllic, pelargonic, butyric, caprylic and capric, and the conclusion was drawn that rancidity is caused by the presence in the fat of the aldehydes corresponding to these acids.

Vincent (382) concluded that when chemical changes occur in butter the glycerides of the insoluble acids are altered to a greater degree than those of the soluble acids. Canzoneri and Bianchini (46) studying the rancidity of olive oil and the oxidation of oleic acid in sunlight and air found nonyllic, azelaic, formic, and dihydroxystearic acids among the products of the oxidation.

Salway (319) oxidized linseed oil at 100° C. in an atmosphere of oxygen and identified acrolein as one of the products. Powick (277) found that all rancid fats and rancid oleic acid gave positive Kreis and peroxide tests. He attributed the Kreis test to the epihydrin aldehyde formed when a rancid fat is brought into contact with concentrated hydrochloric acid. Briggs (28) thought it unlikely that epihydrin aldehyde is formed as believed by

Powick but regarded acrolein as the substance responsible for the Kreis test during the induction period. He suggested also that carotene may be the substance from which the Kreis test arises.

Triebold (370) made a survey of the literature and recognized three general types of fat deterioration, each considered as rancidity, although the processes and end products were different. These three types of deterioration were characterized as oxidative, hydrolytic, and ketonic rancidity. The first of these was considered to be due to the addition of molecular oxygen to unsaturated glycerides with the formation of peroxides which subsequently decompose into aldehydes, ketones, and fatty acids. This type may occur in all edible fats and was regarded as an important type of fat spoilage.

Hydrolytic rancidity is produced by hydrolysis of the glycerides with the liberation of free fatty acids. He regarded this type as important in the spoilage of dairy products.

Ketonic rancidity may develop from fats containing nitrogenous impurities. This results from the action of molds on lower fatty acids with the production of ketones.

It has been thought for a long time that the main factors concerned with fat spoilage are light, air, and moisture. Salkowski (318) believed that the development of rancidity of lard and butter fat was due to the combined activity of oxygen and light, and that it was a direct oxidation process in which the oxygen consumption could be measured. He believed further that the speed of the reaction depended upon the intensity of the light and that little oxygen was consumed in the absence of light. Kerr and Sorber (199) offered the hypothesis that rancidity (oxidative) begins with the entrance of a molecule of oxygen into a molecule of an unsaturated fatty acid, either free or combined, the oxygen molecule attaching itself at the point of double linkage and forming a compound characterized by the peroxide grouping at that point. Tschirch and Barben (377) likewise believed that rancidity starts with the addition of oxygen to the unsaturated acids at the double bond. Subsequently, unstable compounds are formed which break up on hydrolysis to give odorous aldehydes, ketones, and acids. Fierz-David (103) believed that unsaturated fatty acids were split into aldehydes and acids by the action of air, light and water.

Tschirch (376) regarded rancidity as occurring in steps whereby oxygen is added to an unsaturated fatty acid to give a peroxide. The peroxide reacts with water to give an oxide and hydrogen peroxide. Ozone is formed in the presence of hydrogen-peroxide and this reacts with the oxide to give an ozonide. The ozonide is split by the action of water into aldehydes, ketones, and acids. Holm, Greenbank, and Deysher (170) found evidence for the existence of loosely bound oxygen compounds, termed "moloxides," in butter oil. They found ultra violet light to have a powerful effect on the susceptibility of fats to autoxidation. Ellis (96) regarded high dispersion

of the acids as an important factor in the absorption of oxygen by elaidic, oleic, and stearic acids. He found purified, dry sand a good dispersing medium and the cobaltous salt of elaidic acid (0.1 per cent) an efficient catalyst in the autoxidation process. Water and carbon dioxide were evolved during the autoxidation.

Goard and Rideal (124) and Warburg (385) believed that oxidation by ferrous salts resulted from the formation of a peroxide by the ferrous ion, the peroxide acting as an oxidant. Weiland and Franke (393, 394, 395) studied the action of hydrogen peroxide on the oxidation of organic acids in the presence of ferrous salts and concluded that the ferrous ion unites with the substrate thereby making the latter more susceptible to oxidation.

*Butter and Butter Fat*—Garrett and Overman (114) regarded the oxidation of butter fat as not only a simple addition of oxygen at the double bonds but also as involving the oxidation of saturated acids and probably of glycerine. MacLean and Pearce (226) found that hydrogen peroxide with oleic acid in the presence of a copper catalyst brings about oxidation first at the 9th and 10th carbon atoms, and then proceeds further in the 18-carbon chain. The oxidized chain breaks up. Succinic and oxalic were the only dibasic acids isolated. The 8 terminal carbon atoms of the oleic chain are broken off and the remaining part of the chain appears to be completely broken up. The copper salt greatly increased the extent of oxidation.

That tallowy flavor in butter is the result of oxidation of the fat has been shown by numerous workers. Hanus (152) found that when a tallowy flavor developed the acid number increased and the iodine number decreased, but the Reichert-Meissl number was not materially changed. The work of Jensen (192) indicated that olein is the point of attack in the development of tallowy flavor. Siegfeld (329) reported marked changes taking place in the fatty acids, especially in a decrease of volatile insoluble acids and an increase in the solid non-volatile acids. Lea (215) reported that during the early stages of auto-oxidation and prior to the stage of active oxidation, peroxides are formed and these are an indication of the relative ease with which a fat will produce tallowy flavor. He also reported that bleaching occurred at an early stage in the oxidation. Clavel (49) reported a relationship between the drop in iodine number of fats during storage and the absorption of oxygen causing oxidative rancidity.

Stebnitz and Sommer (339, 340) studied the effect of the composition of butter fat on its susceptibility toward oxidation. They found the stability of a fat toward oxidation bore an inverse relation to the degree of unsaturation of the fat, with linoleic acid content rather than oleic acid the principal governing factor. When cows received grass as a part of their ration the fat became less saturated and more susceptible to oxidation. These workers (338) found that a tallowy flavor and peroxide formation began at about the same time and that considerable loss of color had already occurred.

Sommer (335) reported that trimethylamine was the immediate cause of fishiness in butter. He believed this flavor to be the result of oxidation of the lecithin in the butter. That fishiness is the result of oxidation of the lecithin has been verified by the work of Holm *et al.* (172) and Jensen and Ritter (193), but the steps whereby this oxidation takes place have not been entirely established.

*Milk and Cream*—As early as 1916 Guthrie (141) had recognized a metallic flavor in cream and his observation had led him to believe that it was caused by factors other than direct contact with metals. He observed further that cream having only a slight metallic flavor yielded buttermilk which was very metallic in flavor after churning in a glass churn. He was unable to establish a relationship between the fatty acids, caprylic, palmitic, stearic, oleic or propionic and the metallic flavor. In 1932 Tracy (363) reported "cappy" or "tallowy" flavor in milk to be the result of oxidation of the milk fat. This view was supported the following year by the report of Kende (198) who found a decrease in iodine number of butter fat when an "oleagenous" or oily flavor developed in the milk. Mohr and Wellm (246) stated that "emery" flavor is similar to but not identical with oily, tallowy, or rancid flavors. They reported that the unknown compound causing emery flavor was soluble in the fat phase and hence carried into the butter. Dahle (66) reported that butter fat was the substance affected when oxidized flavor develops in milk. He found that the iodine number decreased in proportion to the degree of flavor present.

In 1935 Thurston and Barnhart (358) reported an oxidized flavor in the buttermilk from churned sweet cream. This product was known to be high in phospholipids. In the same year Thurston, Brown and Dustman (359) found that oxidized flavor was pronounced in the cream, skim milk, butter and buttermilk, but that only a faint trace of the flavor was found in the butter fat washed by continuous reseparation and dilution with water. When the washed butterfat was redispersed in skim milk no oxidized flavor could be detected. Likewise, they were enable to develop an oxidized flavor in this product by methods ordinarily used to cause the development of this flavor defect. These workers caused a tallowy flavor to develop in butter oil by bubbling oxygen through it at 75° C. When they redispersed this oxidized butter oil by homogenization into the skim milk, a typical tallowy flavor resulted. However, the flavor was unlike the characteristic oxidized flavor of milk. These authors believed that lecithin rather than butterfat was affected when oxidized flavor developed in milk. In a later work (360) they explained the inhibiting effect of agitation of cold milk on the development of oxidized flavor as being the result of partial transfer of lecithin from the fat globule surface to the plasma. They considered this as further evidence that lecithin was the constituent affected. Dahle (65) reported that cream and pure butter oil mixed with the skim milk of certain cows de-

veloped an oxidized flavor, but that the cream often reacted more quickly than the butter oil. He considered this as evidence that the phospholipids may react before the fat itself. The phospholipids of milk are readily susceptible to oxidation.

Brown, Dustman, and Thurston (32) in 12 trials during the winter months of two successive years found no measurable change in the iodine number of milk fat from oxidized and normal milk. They showed by calculation that one would not expect to obtain any difference in the iodine number of milk fat as a result of oxidation of the double bond in oleostearo lecithin because of the relatively small amount of lecithin present.

In a more recent work Swanson and Sommer (347) were unable to find any difference in the iodine number of butterfat from normal and oxidized flavored milk. These investigators criticized the calculation of Brown, Dustman and Thurston on the basis that it had been shown that lecithin contains two oleic acid radicals. However they found from the determination of the iodine number of the phospholipid fraction that the development of oxidized flavor is accompanied by a marked decrease in the iodine number of the phospholipid fraction. They concluded that the development of oxidized flavor in milk, catalyzed by copper, is primarily due to the oxidation of the phospholipid fraction and that the oxidation of the unsaturated fatty acids in this fraction is not complete. The indications were that one molecule of an unsaturated fatty acid, undoubtedly oleic acid, remained unoxidized.

Dahle and Palmer (78) considered spontaneous oxidized flavor, *i.e.*, without copper catalysis, to be due to the oxidation of the phospholipid fraction of the fat globule membrane and the butterfat.

Roland and Trebler (311) studied the effect of fat content on oxidized flavor in milk and cream. They reported that mechanical separation of milk produced a marked decrease in its sensitivity to copper-induced oxidized flavor as evidenced by tests on reconstituted milk made by recombining cream and skim milk. They suggested that the removal of lecithin or related substances by the separator or changes in their distribution between the fat and aqueous phase may be responsible for the decreased sensitivity.

Josephson and Doan (196) reported that they were able to develop a typical oxidized flavor by heating a suspension of pure phospholipids in water in the presence of copper. A protein suspension similarly prepared developed a different flavor when heated in the presence of copper. A suspension of phospholipid and protein combined, oxidized readily without heat or copper but became much more tallowy (oxidized) when heated, treated with copper, or when subjected to both treatments.

In 1936 Greenbank (133) reported that some constituent of milk is readily oxidized since the oxidation occurs at 5° C. in an atmosphere of 2.5 per cent oxygen. He excluded from consideration fat, casein, lactose, and albumen, and stated that if lecithin was the factor involved, as reported by

Thurston, *et al.*, all milks should develop oxidized flavor since they all contain lecithin. Apparently the problem was much more involved than he believed at that time. Greenbank presented a theory in which he visualized the flavored product as an intermediate step in an oxidation reaction, the end product being flavorless. At the present time the literature on oxidized flavor does not reveal cases in which oxidized flavored products have lost their flavor due to continued oxidation.

The present status of the literature indicates that tallowy flavor of butter is probably the result of oxidation of the fat, this oxidation beginning at the surface and probably working toward the center of the product. Likewise, the evidence points to a fishy flavor of butter as being the result of an oxidation process. In this case lecithin is probably the parent substance of trimethylamine, the latter being the immediate cause of fishy flavor. The trend in the literature at the present time seems to point to the phospholipid fraction as the source of oxidized flavors in milk and cream. However, as a result of differences in terminology of flavors many apparently conflicting lines of evidence have been reported.

### XIII. METHODS OF PREDICTING AND DETECTING ONSET OF OXIDATION

Holm and Greenbank (166) reported in 1922 that oleic acid exposed to oxygen for some time gave the characteristic tallowy odor and proportionate peroxide and Kreis tests. In a later work (165, 167) they measured the amount of oxygen absorbed by a fat during oxidation and studied acidity, iodine number, iodine liberated from potassium iodide in 24 hours, and the Kreis test as means of detecting rancidity. Of these tests only the Kreis test gave results that detected small differences in the degree of oxidation. Bevis (23) found that aeration caused bleaching, the development of a positive Kreis test, and a decrease in the iodine number. She observed an increase in free fatty acids but this had no relation to the Kreis test.

Holm and Greenbank (168) studied the relation of the Kreis test to oxygen absorption by linoleic and ricinoleic acids. These acids absorbed oxygen and gave a positive Kreis test, the colors developed were less intense than with oleic acid. Linoleic and ricinoleic acids gave but faint tallowy odors even after large amounts of oxygen were absorbed. They (137a) describe an apparatus for determining the oxygen absorption by fats. Nagel and Von Have (257) using an oxygen uptake procedure demonstrated a catalytic effect of copper oxide on drying and non-drying oils. Tschirch and Barben (377) reported that rancid fats gave the Kreis test for deterioration and showed peroxides present. Inichof and Schosldrine (186) reported that a determination of the amount of aldehydes present is the only means of precisely estimating the rancidity of butter. They described a method for their determination.

Davies (81) reported a test for forecasting the deterioration of butter by means of oxidation potentials as determined by methylene blue.

Lucas *et al.* (223) checked the scores of butter against amino nitrogen and total nitrogen, acid number, iodine number, Reichert-Meissl number and the Kreis test. After six months storage each sample was tested for trimethylamine. They found no relationship between these tests and the keeping quality as determined by score except in the case of the Kreis test. Butter with a fishy flavor after storage for six months showed a positive trimethylamine test. Briggs (28) reported that the acid, iodine and peroxide values remained practically unchanged during the induction period. The acid value failed to show a close relation to the absorption of oxygen, there being a distinct lag indicating that the production of acid is a secondary process. He concluded that the acid value was not a satisfactory means of detecting the progress of oxidation. The iodine value was found to decrease nearly in proportion to the absorption of oxygen. He concluded that although the determination of iodine number gives no indication of any changes during the induction period, it does give a fairly satisfactory guide to the progress of later oxidation. The Kreis test was found to be quite sensitive.

Kobert (204) reported that only compounds containing an allyl group or a substituted allyl group were capable of forming red condensation products with phloroglucinol. Ginzberg and Fomina (123) proposed a new constant "oxygen number," for fat investigation. The oxygen number is defined as the number of mg. of oxygen (from  $\text{KMnO}_4$ ) required to titrate a fat. It is similar to, but not identical with, the iodine number.

Triebold and Bailey (371) found little or no relationship between the iodine number and the keeping quality of shortening. There was a slight tendency for those samples showing lower iodine numbers to have better keeping quality.

Taufel and Thaler (350) reported a new analytical method for the determination of ketones in rancid fats. Aspegren (12) studied the changes in shortening and oils by heating them in an oven at 145° F. This treatment over a period of 106 days increased the nitrogen content and decreased the iodine number. Taufel (349) and Wheeler (397) have reviewed the tests for fat spoilage. The latter developed a convenient method for the determination of peroxides. This is not satisfactory as a single test in the early stages but after appreciable oxidation has occurred it indicates how far the oxidation has proceeded. Wheeler found a high peroxide value always accompanied by a high Kreis test. Frehden (108) likewise has described a new test for fat spoilage but its sensitivity has not been determined. Ritter (292a) developed a test for copper in butter by use of the peroxide reaction. The copper content of butter is closely associated with keeping quality. Joyner and McIntyre (197) used the oven test as an index to keeping quality of fats. Closely paralleling this is the holding test for butter as reported by Jacobsen (190) and Parsons (273). Under this test the butters are held at room tem-

perature for a short period, whereby the rate of decomposition is greatly accelerated. Ritter and Nussbaumer (301, 304) reported a method for the determination of keeping quality based upon the length of time required for the peroxide number as developed by Wheeler to reach a value between 5 and 10. Beyond this range the value increases very rapidly.

Trout and Sharp (375) found taste a reliable method for the detection of oxidized flavor in milk.

Many chemical tests have been proposed as measures of the rapidity with which oxidation takes place and these have met with varying degrees of success. Among them the Kreis test and the peroxide number, however, appear to be the most sensitive and of greatest value in detecting the onset of oxidation.

#### XIV. ANTIOXIDANTS

*Early Work*—The original work on antioxidants was probably inspired by the finding that certain substances accelerated the rate of oxidation of certain materials. Bevis (23) found that oleic acid added to beef fat promoted the development of rancidity. Glycerin had no effect. Fridericia (110) reported that when certain animal fats, such as lard or hydrogenated whale oil, were mixed in rations containing butter as a source of vitamin A the animals suffered from vitamin A deficiency. Greenbank and Holm (137) observed the acceleration of oleic acid on the rate of oxidation of fats. Moureu and Dufraisse (252) studied the catalytic properties of iodine and its compounds on the auto-oxidation of acrolein. In each case an appreciable positive or negative effect was observed, but the nature of the action could not be predicted.

Smith and Wood (332) studied the effect of inhibiting agents in the oxidation of unsaturated organic compounds. Over 100 chemicals were tried using the oxygen absorption method. They classified the retarding agents in order of their effectiveness. These workers expressed two hypotheses: 1. The antioxidant, being basic, combines with the acidic products of oxidation and prevents them from acting as autocatalysts toward oxidation. 2. The triple-bond N atom with 2 partial valences, or elements with free valences, forms intermediate compounds with the easily oxidized ethenoid carbon. This temporary compound controls the rate of reaction for a definite, but limited period of time. Holm, Greenbank, and Deysher (170) presented evidence of the existence of loosely bound oxygen compounds in butter oil. They considered these substances, termed "moloxides," as responsible for oxidation in vacuo. They reported also a powerful retarding action of OH groups in a position analogous to that of the second unsaturated bond in linoleic acid.

Husa and Husa (185) reported that addition of 0.5 per cent of hydroquinone to lard reduced the rate of development of rancidity about 50 per cent. No effect was found from the following compounds on the rate of development of rancidity; salicylic acid, acetyl-salicylic acid, beta-naphthol,

liquefied phenol, dl-alanine, pyrogallic acid and resorcinol. Clark (48) found that from 0.5 to 1.0 per cent of phenyl-a-naphthylamine added to such oils or waxes as those used in electric insulation prevented oxidation. Matill and Crawford (236) suggest that the mere presence of double bonds is of less moment for the process of oxidation than is that of substances which initially either accelerate or retard the reaction. The sterols of corn oil were found to greatly prolong the induction period of lard, but acetylated sterols reduced the induction period. Moureu *et al.* (253) prefer the use of the term "anti-oxygen" to antioxidant because they claim that an article, which is to be protected against oxygen, after having been subjected to the action of air, will need a greater proportion of anti-oxygen to make up for that which will be destroyed by the peroxides already formed.

Hilditch and Paul (159) reported basic compounds in linseed oil and cottonseed meal. The antioxygenic activity was suppressed by treatment with anhydrous HCl and partly restored by neutralization with sodium methoxide. The identity of the basic compound was not determined, but they believed it to be a basic oxygen rather than a basic nitrogen compound. Matill (235) made a review of the antioxidants and autoxidants of fat. After studying a large number of compounds his observations indicate that antioxygenic capacity of phenols resides in two hydroxyl groups in either the ortho or para configuration; when these were in the meta position the compound was inactive. The hydroxyls were ineffective unless attached directly to the ring; the fully hydroxylated inosite is inactive. In the naphthols one hydroxyl group is sufficient and in keeping with its accepted behavior, a-naphthol has the character of an ortho compound and is much more effective as an antioxidant than b-naphthol; quinone is effective and b-naphthoquinone is even more so but the alpha form is inactive. Stiepel (341) also found naphthol effective. Matill discusses these facts in relation to the more recent theories of the electronic structure of the benzene ring and autoxidation. A number of sterols of animal origin and sitosterol from wheat, corn, and lettuce were all inactive. The existence of pro- and antioxygenic substances among the non-saponifiable constituents of natural fats and oils suggests that some of these may be concerned with the physiological action of the fat-soluble vitamins. Baumann and Steenbock (19) reported that pure vitamin A and pure carotene were destroyed by ultra-violet light in one-half hour whereas the crude product was stable during exposure for five hours. Briggs (27) reported antioxygenic effect for curd in butter whereas humidity, glycerol, triolein, lactose, iodine, potassium iodide and pasteurization had little or no effect.

Monalgalm and Schmidt (247) reported that vitamin A was found to completely inhibit oxygen uptake of linoleic acid for some hours but that it loses inhibiting power when the vitamin is destroyed by oxidation. Likewise, the presence of carotene was shown to strongly inhibit oxidation during the first

few hours during which time it becomes bleached (presumably oxidized), and then it increases the oxygen uptake. Koenig (206) found that carotenoid pigments inhibit rancidity in fats and Greenbank and Holm (139) reported carotene an antioxidant for acids but not for glycerides. Newton (259) reported that carotenoid pigments act as antioxidants and that these antioxidant properties carried over into finished bakery products. In contrast to this Bradway and Matill (26) and Olcovich and Matill (265) report that carotene is pro-oxygenic but that crude carotene may be antioxygenic due to an associated inhibitor. In the latter work these investigators found that carotene in autoxidizable fat shortened the induction period and concluded that carotene is a pro-oxidant.

Guillaudeau (140) reported that cyanamide compounds added to soap chips inhibit oxidation. Murrill (256) reported phenyl phenolate to have antioxidant properties. Yamaguchi (410) found antioxidant properties for copper oleate and manganese linoleate. Palit (269) reported that proteins and carbohydrates retard fat oxidation. Roller (312) stated that not all samples of wheat germ oil act as antioxidants for fat, oils, and food rations. The activity was not dependent upon the amount of lecithin, sitosterol, unsaponifiable matter or free from fatty acids present. He believed the activity to be due to free OH groups either on the glycerol or on the ricinoleic acid which the oil was thought to contain. Salzberg (320) reported antioxidant activity for catechol monododecyl ether or other suitable phenols of the benzene series having at least one alkoxy group in position o- or p- to a hydroxyl group and containing 12 carbon atoms in such alkoxy group. Greenbank (132) found the treatment of oils or fats with a small proportion of an unsaturated polybasic aliphatic acid such as maleic, aconitic, fumaric, citraconic or itaconic acid or derivatives such as maleic anhydride or ethyl or sodium maleate, to be effective in inhibiting oxidation. In a later work Greenbank and Holm (139) found hydroquinone, phthalic acid and maleic acid good antioxidants. Maleic acid was the best of the three. Olcott (261) found pyrogallol, hydroquinone, pyrocatechol, hydroxyhydroquinone and apionol excellent antioxidants. The 1, 3, and 1, 8 naphthalenediols were effective whereas the 1, 4 derivative was inactive. The esterification and alkylation of one or more of the hydroxyl groups destroyed or greatly reduced antioxygenic activity. Side chains of the benzene nucleus reduce the activity of hydroquinone. The quinones possess slight activity and 1, 4 cyclohexanediol and saligenin were inactive. Maleic, tartaric and citric acids were inactive.

Coe and LeClerc (55) found maleic and phthalic acids, hydroquinone, pyrogallol, pyrocatechol and guaiacol to act as antioxidants. Lea (217) reported Na-maleate, NH<sub>4</sub>-lactate, Na-tartrate, Na-glycolate, and Na-lactate as moderate antioxidants and glycine, asparagine, Na-citrate, and Na-malonate as powerful antioxidants.

In 1935 Evans (100) found that when mixed with cotton seed oil, lecithin (probably a mixture of lecithin and cephalin) greatly retarded peroxide formation. It was found more effective than diphenylguanidine, hydroquinone and KCN. He found the antioxidant property of lecithin destroyed by heating to 65° C. Sitosterol was also shown to inhibit peroxide formation. He explained the antioxidant properties of palm kernel oil on the basis of its sitosterol content. Holmes *et al.* (173) reported hydroquinone and lecithin as antioxidants for vitamin A in halibut liver and cod liver oils. Lecithin was found more effective than hydroquinone at 96° C. Koenig (206) found lecithin to inhibit the development of rancidity of butter. Olcott and Matill (264) reported that purified lecithin was not an antioxidant but that purified cephalin was. Ritter and Nussbaumer (303) found lecithin and particularly cephalin obtained from plant lecithin, to have strong antioxidantizing effect.

Sabalitschka and Bohm (317) reported that the oxidation of organic substances readily liable to atmospheric oxidation is prevented by incorporation of substances of the formula  $\text{RCOOR}'\text{N}(\text{R}'')\text{R}'''$ , where R is hydrogen, alkyl, alkylene or aryl, which may be substituted by OH, hydroxyalkyl, an alkyl or  $\text{NH}_2$ , R' is alkylene with not more than 4 carbon atoms, R'' is alkylene with not more than 3 carbon atoms and R''' is hydrogen or alkyl with not more than 3 carbon atoms. Hinsberg and Nowakowski (162) found that many porphyrins inhibit autoxidation of linseed oil. The effect was related to the number of carboxyl groups in the molecule. Kochling and Taufel (205) studied the role of maltol in fat spoilage. It was found to exert antioxidant action only under highly acid conditions. French, Olcott, and Matill (109) found a prolongation of the induction period by fractions of unsaponifiable lipids of wheat germ oil and palm oil.

Hamilton and Olcott (149) reported that from experiments with antioxidants phenolic inhibitors and inhibitos cause no change subsequent to the end of the induction period, but exert their effect solely by inhibiting the formation of the initial monoxide and are then entirely destroyed before the start of rapid oxidation. Olcott and Matill (263) studied antioxidants and autoxidation of fats. They classified the inhibitors into three groups: 1. acid type, 2. inhibitol and hydroquinone, and 3. other phenolic inhibitors. In general any type one inhibitor used with any type 2 or 3 compound prolonged the induction period to a much greater extent than would be expected from a summation of the effects of each used alone.

Olcott and Emerson (262) found the tocopherols to have antioxidant properties in lard. Maveety (238) found the residue from the distillation of spice oils to have antioxigenic properties. The substances were not isolated. Takahashi and Masuda (348) found the phenol compounds in incompletely burned oak smoke to have antioxigenic properties. Ritter and Nussbaumer (301) reported antioxidative properties for hydroquinone.

Coun and Asnis (58) found that dusting freshly prepared potato chips with oat flour retarded peroxide formation. Likewise treating parchment paper wrapper with oat flour definitely retarded oxidation of lard. Lowen, Anderson and Harrison (221) found oat flour to retard the initial peroxide formation in halibut and salmon oils.

*Butter and Ice Cream*—Koenig (206, 206a) found that parchment paper wrapper treated with oat flour and a hexane extract of oats (avenol) used in butter, had a protective action against the development of rancidity. Dahle and Josephson (74) found that avenex-treated parchment paper was beneficial in retarding flavor defects at 45° F. but of little benefit as long as butter remained in storage at -15° F. In another study they found water extract of avenex improved the keeping quality of butter without injuring the flavor or incorporating sediment into the butter. Dahle (68) reported that oat flour in butter delayed not only the development of tallowiness but also such flavors as stale and cheesy. He suggested that these flavors may be of an oxidative nature. Ritter and Nussbaumer (303) found avenex to have antioxidant properties as did Corbett and Tracy (56) who reported a beneficial effect on the development of oxidized flavors in butter. The use of avenized parchment paper retarded surface flavors and improved keeping quality.

Mueller and Mack (255) found that 0.25 per cent of oat flour in ice cream had antioxidative properties and delayed the development of off-flavors. They found 0.5 per cent still more effective. Brown (36) likewise found 0.5 per cent effective. Weckel (389) recommended the use of not more than 0.3 per cent avenex for vanilla ice cream and up to 0.5 per cent for strawberry ice cream. Dahle and Josephson (72, 73) reported 0.3 per cent not quite sufficient to completely inhibit oxidized flavor development in strawberry ice cream beyond 4 weeks time. However, 0.5 and 0.7 per cent proved an effective antioxidant. Maack and Tracy (224) and Burke and Newman (42) found 0.5 per cent of avenex an ample antioxidant for preserving the fresh flavor in ice cream.

Bird, Ross, Iverson, Ause and Willingham (24) reported that the higher the iron content of ice cream the less the tendency for it to develop oxidized flavors. They believed the iron probably combined in the ferrous form and served as an antioxidant.

*Milk and Cream*—Ritter (291) obtained antioxidative properties from hydroquinone, metol and ascorbic acid when used for tallowy flavor in milk. Ritter and Christen (296) reported from 5 to 7 per cent of hydroquinone in a bacteria culture which was known to prevent a tallowy flavor in milk. Chilson (47) found that ascorbic acid and hydroquinone when added directly to milk prevented oxidized flavor development. Greenbank (133) also reported the beneficial effect of added ascorbic acid on milk. Dahle and Palmer (78) and Dahle (67) in addition to confirming these findings on

ascorbic acid, substantiated the effect of hydroquinone and added oat flour as antioxidants for milk. Maleic acid and carotene, in the quantities used, were found to be ineffective. Guthrie, Hand and Sharp (143) found that the use of paper bottles markedly decreased the effect of sunlight on the oxidation of ascorbic acid and on the production of off-flavors. Garrett (111) found that oat flour exhibited definite antioxidative properties in retarding the development of oxidized flavor in milk as induced by copper contamination or exposure to sunlight. Weckel (389) recommended the use of 2 per cent avenex for controlling stale flavor in storage cream.

Deco (87) reported that boiling milk in large lots for an hour or more in contact with air, as practiced in some Belgian hospitals, destroyed 40 to 65 per cent of the vitamin A and 20 to 25 per cent of the carotene. Both of these substances have been reported as having certain antioxidative properties.

Anderson (4) reported that the use of pancreatic enzyme in milk prevented the development of oxidized flavor whether the difficulty is due to metal contamination or to naturally high susceptibility of the milk. He believed the pancreatic enzyme to be specific for the prevention of the development of oxidized flavor and that it acted as an antioxidant.

Apparently there are a number of antioxidants which will prevent the development of oxidized flavor in milk and dairy products. The use of these in milk is largely restricted because of laws in most states which prohibit the addition of any non-milk ingredient to milk.

#### SUMMARY

Of all the factors concerned in bringing about oxidative changes in dairy products metallic contamination, particularly by copper, is at present the most important. The control of copper contamination in processing becomes increasingly more important as the sanitary quality of the products is improved. However, not all milk must have copper contamination to develop an oxidized flavor. Fortunately, the percentage of milk which develops oxidized flavor spontaneously is relatively small and when this milk is mixed with normal milk it remains normal in flavor. For this reason it is not of as great commercial importance as is milk susceptible to metal-induced oxidized flavor.

Oxidized flavor in milk has been shown to be associated with milk of low bacterial count. The growth of bacteria in the milk, either by using up the oxygen or by reduction of the potential, render milk non-susceptible even in the presence of copper.

Oxidized flavor in milk was originally believed to be the result of oxidation of the fat catalyzed by copper and brought about through the action of an enzyme. The recent trend in literature points to the phospholipid fraction as the source of the flavor and recent work on cooked flavor questions seriously the action of an enzyme in bringing about the defect.

Grass feeding has been shown to reduce the susceptibility of milk to oxidized flavor even though the milk fat is made more susceptible to ordinary chemical oxidation. This is explained on the basis of reducing substances in the feed or in the milk, or in both. Various investigations have shown that ascorbic acid and carotene in the feed tend to reduce the susceptibility of the milk. Also a number of antioxidants have been demonstrated to have protective qualities. The mechanism whereby an oxidized flavor develops has not been completely demonstrated nor has the mechanism whereby the various factors exert their effects.

Tallowy flavor in butter appears to be the result of metal-induced oxidation of the fat, the point of attack probably being the double bonds in the oleic and linoleic acid radicles.

Light and oxygen have been demonstrated to be important factors favoring oxidative changes. Oxidation will take place in the absence of light but the rate of reaction is slow. The source of oxygen may be either the free or the combined form.

Both strong acid and alkaline reactions have been shown to favor oxidative changes. Over-neutralization is known to favor oxidative changes while butter made from high acid cream in the presence of copper contamination and salt is prone to become fishy upon storage.

Both salt and moisture appear to play a role in the development of oxidative changes but they are of minor importance when compared to metallic contamination.

Temperature is important only as a regulator of the rate of oxidative change. As the temperature increases the rate of oxidative change increases, all other factors being constant. Low temperature storage favors a slow rate of oxidative change.

Development of tallowy or oxidized flavor in ice cream is undoubtedly an oxidative change. In this product, however, we have the possibility that the oxidative changes may affect either the phospholipid fraction or the butterfat or both. The present work points toward fat as the substance oxidized. If the present trend of research on oxidized flavor of milk continues, a reexamination of tallowy flavor in ice cream would seem desirable.

The elimination of copper contamination is one of the major problems confronting the dairy industry today as the flavor problems resulting from chemical reactions have a copper history in the vast majority of cases. If these problems are to be eliminated by removal of the cause, it will be necessary for copper to be eliminated from all surfaces with which milk comes in contact because of the extremely small amount of copper required to develop the flavor in many cases.

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## REPORT OF THE STUDENTS' NATIONAL CONTEST IN JUDGING DAIRY CATTLE

SAN FRANCISCO, CALIFORNIA, OCTOBER 21, 1939

Seventeen teams competed in the Dairy Cattle Judging Contest held on Treasure Island in conjunction with the National Dairy Show and the Golden Gate International Exposition. The Iowa State College team won first honors on all breeds and Robert Lage of Iowa State was high individual of the contest.

Breed honors were divided, with the Texas A. & M. College team ranking first in Ayrshires and Brown Swiss. A. A. Price, of the Texas A. & M. team, was high individual in judging Ayrshires, while individual honors on Brown Swiss went to D. C. Marsh of the same team.

The Guernsey trophy was won by the team from the University of Missouri with individual high score being made by A. A. Price of Texas A. & M. College.

In judging Holsteins, South Dakota State College won first in team standings, while Paul Astleford of Oregon State College was high ranking individual.

First rank in team standings in Jersey judging was won by the University of Wisconsin. T. H. Blosser of Purdue University was high ranking individual and won the \$400 Research Scholarship awarded by the American Jersey Cattle Club.

The ten high ranking individuals and the team rank for the various divisions of the contest are indicated in the following lists:

### ALL BREEDS *Individuals*

1. Robert Lage, Iowa State College
2. A. A. Price, Texas A & M College
3. E. Halbach, University of Wisconsin
4. Quinten Syse, University of Wisconsin
5. Paul Astleford, Oregon State College
6. Russel Pfeiffer, University of Nebraska
7. T. H. Blosser, Purdue University
8. Alva Clark, University of Missouri
9. Gilbert Walker, Oklahoma A & M College
10. Raymond French, University of Illinois

### Teams

- |                                |                                       |
|--------------------------------|---------------------------------------|
| 1. Iowa State College          | 10. University of Illinois            |
| 2. Oklahoma A & M College      | 11. University of Tennessee           |
| 3. Texas A & M College         | 12. University of Minnesota           |
| 4. University of Wisconsin     | 13. South Dakota College              |
| 5. University of Nebraska      | 14. New Mexico College of Agriculture |
| 6. University of Missouri      | 15. Kansas State College              |
| 7. Purdue University           | 16. Pennsylvania State College        |
| 8. Oregon State College        | 17. University of Georgia             |
| 9. Texas Technological College |                                       |

**AYRSHIRES***Individuals*

1. A. A. Price, Texas A & M College
2. Jack Hancock, Texas Technological College
3. Chas. Bennett, University of Illinois
4. Tom Miles, University of Tennessee
5. Dallas Rierson, New Mexico College of Agriculture
6. Donald Jordan, University of Minnesota
7. Alva Clark, University of Missouri
8. Eugene Halbach, University of Wisconsin
9. Gilbert Walker, Oklahoma A & M College
10. W. C. Foster, Texas A & M College

*Teams*

- |                                      |                                |
|--------------------------------------|--------------------------------|
| 1. Texas A & M College               | 10. University of Illinois     |
| 2. University of Minnesota           | 11. Oregon State College       |
| 3. University of Nebraska            | 12. University of Missouri     |
| 4. Purdue University                 | 13. Pennsylvania State College |
| 5. University of Tennessee           | 14. University of Georgia      |
| 6. Oklahoma A & M College            | 15. Iowa State College         |
| 7. University of Wisconsin           | 16. South Dakota State College |
| 8. New Mexico College of Agriculture | 17. Kansas State College       |
| 9. Texas Technological College       |                                |

**BROWN SWISS***Individuals*

1. D. C. Marsh, Texas A & M College
2. Fred Giesler, University of Minnesota
3. Quinten Syse, University of Wisconsin
4. A. A. Price, Texas A & M College
5. Claire Wemer, Iowa State College
6. Willis Jones, University of Georgia
7. Elmont Honeu, Texas Technological College
8. Jack Hancock, Texas Technological College
9. Alva Clark, University of Missouri
10. Paul Astleford, Oregon State College

*Teams*

- |                                      |                                |
|--------------------------------------|--------------------------------|
| 1. Texas A & M College               | 10. University of Minnesota    |
| 2. Texas Technological College       | 11. University of Illinois     |
| 3. Purdue University                 | 12. Oregon State College       |
| 4. University of Missouri            | 13. University of Nebraska     |
| 5. New Mexico College of Agriculture | 14. University of Georgia      |
| 6. Iowa State College                | 15. Kansas State College       |
| 7. University of Tennessee           | 16. Pennsylvania State College |
| 8. University of Wisconsin           | 17. South Dakota State College |
| 9. Oklahoma A & M College            |                                |

**GUERNSEYS***Individuals*

1. A. A. Price, Texas A & M College
2. T. H. Blosser, Purdue University
3. Richard Schuckenbrock, University of Missouri
4. Gilbert Walker, Oklahoma A & M College
5. George Smith, University of Illinois
6. Wm. H. Winner, Kansas State College
7. Tom Miles, University of Tennessee
8. Otto Pfeiffer, Jr., University of Nebraska
9. Russell Pfeiffer, University of Nebraska
10. B. I. Iffit, Pennsylvania State College

*Teams*

- |                                      |                                |
|--------------------------------------|--------------------------------|
| 1. Oklahoma A & M College            | 10. Iowa State College         |
| 2. Texas A & M College               | 11. University of Georgia      |
| 3. University of Missouri            | 12. University of Wisconsin    |
| 4. Texas Technological College       | 13. Purdue University          |
| 5. Kansas State College              | 14. University of Illinois     |
| 6. University of Nebraska            | 15. University of Minnesota    |
| 7. University of Tennessee           | 16. Pennsylvania State College |
| 8. South Dakota State College        | 17. Oregon State College       |
| 9. New Mexico College of Agriculture |                                |

**HOLSTEINS***Individuals*

1. Paul Astleford, Oregon State College
2. Eugene Halbach, University of Wisconsin
3. Raymond French, University of Illinois
4. Clifford Manry, Oklahoma A & M College
5. Elmer Dent, Oregon State College
6. Ray Johnson, University of Minnesota
7. L. G. Duncan, New Mexico College of Agriculture
8. Jacob Stinson, Iowa State College
9. George Smith, University of Illinois
10. Melvin Jensen, South Dakota State College

*Teams*

- |                                   |                                 |
|-----------------------------------|---------------------------------|
| South Dakota State College        | 10. Pennsylvania State College  |
| Oregon State College              | 11. Texas Technological College |
| University of Illinois            | 12. University of Nebraska      |
| Iowa State College                | 13. University of Tennessee     |
| New Mexico College of Agriculture | 14. Texas A & M College         |
| Oklahoma A & M College            | 15. University of Missouri      |
| Kansas State College              | 16. Purdue University           |
| University of Minnesota           | 17. University of Georgia       |
| University of Wisconsin           |                                 |

**JERSEYS***Individuals*

1. T. H. Blosser, Purdue University
2. Robert Lage, Iowa State College
3. John Liggett, Oklahoma A & M College
4. Eugene Halbach, University of Wisconsin
5. Otto Pfeiffer, Jr., University of Nebraska
6. Quinten Sysc, University of Wisconsin
7. Phillip E. Kizer, University of Missouri
8. Claire Wener, Iowa State College
9. Clayton Fox, Oregon State College
10. Willis Jones, University of Georgia

*Teams*

- |                            |                                       |
|----------------------------|---------------------------------------|
| 1. University of Wisconsin | 10. University of Illinois            |
| 2. Iowa State College      | 11. Kansas State College              |
| 3. Oklahoma A & M College  | 12. Texas A & M College               |
| 4. University of Missouri  | 13. South Dakota State College        |
| 5. University of Nebraska  | 14. Texas Technological College       |
| 6. Purdue University       | 15. Pennsylvania State College        |
| 7. Oregon State College    | 16. University of Georgia             |
| 8. University of Tennessee | 17. New Mexico College of Agriculture |
| 9. University of Minnesota |                                       |



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## FURTHER OBSERVATIONS ON BASIC VISCOSITY OF ICE CREAM MIXES AND A SIMPLIFIED PROCEDURE TO OBTAIN IT\*

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The viscosity of an ice cream mix which is quiescently aged reaches a maximum value which may be broken down by mechanical agitation to a lower constant minimum viscosity. Leighton and Williams (1) studied this phenomenon and named the two viscosities "apparent" and "basic." They reduced ice cream mixes from apparent to basic viscosities by agitating for 60 minutes in a completely filled ice cream freezer. The measuring of basic viscosities of ice cream mixes is now a common experimental procedure. Dahlberg, Carpenter, and Hening (2) used a tightly sealed fruit jar with a Daisy churn agitator to secure basic viscosity. This method has the advantages of small samples of mix and short periods of agitation as compared to that used by Leighton and Williams (1). Whitaker (3) constructed an apparatus to break apparent viscosity down to a constant basic value. Hening (4) has shown in this laboratory that if the aged ice cream mix is rehomogenized several times at low pressures the basic viscosity is lower than that obtained by the Whitaker device. This he attributed to a more thorough breaking up of the fat clumps by the homogenizer.

The fact that fat clusters in milk and cream affect viscosity was shown in 1896 by Babcock and Russell (5).

To further bear out this fact the following quotation is taken from an abstract of a paper written by Weissenberger (6) "Dispersoids, more practically emulsoids whose concentration exceeds a certain limit tend to form secondary aggregates of the primary particles of the disperse phase. These structures of higher orders are easily broken down by mechanical agitation, by shaking or forcing the solution through capillaries. If the viscosity of such an emulsion is measured in an Ostwald viscometer it is bound to fall

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off with successive passages through the capillary until a constant value is obtained, the magnitude of which depends upon the size of the capillary. If the dispersoid is then allowed to rest, the viscosity slowly increases again as the structures of higher order again are formed."

Mortensen (7) was perhaps the first to state that homogenization produced large fat clusters. Leighton and Williams (1) have shown that if an aged mix is repeatedly run through an Ostwald viscometer, the time required for the flow of the mix gradually decreases to a constant value that is dependent on the instrument itself. This is in agreement with Bateman and Sharp (8) who obtained the same results for whole milk. They further state that the reduction of viscosity was related to the breaking up of fat clumps.

In addition to the breaking up of fat clumps, it is known that agitation will break up the gel structure in ice cream mixes formed by gelatin, resulting in a reduced viscosity.

With the fact in mind that the basic viscosity as related to apparent viscosity of an ice cream mix is affected chiefly by the size and number of fat clusters and by the gel structure, a study was undertaken to determine whether a hand emulsifier is more efficient and more practical than the Whitaker device (3) in reducing apparent viscosity to basic viscosity.

#### EXPERIMENTAL METHOD

Preliminary trials on an ice cream mix, containing 12 per cent milk fat, 12 per cent milk solids-not-fat, 15 per cent sugar, and 0.4 per cent gelatin, pasteurized at 65.5° C. (150° F.) for 30 minutes, and homogenized with a two-stage valve at 2500 and 500 pounds pressure per square inch at pasteurization temperature and aged at 40° C. (39.2° F.) for 18 hours, showed that the hand emulsifier reduced the mix to a lower basic viscosity than the Whitaker device. The ice cream mix was emulsified twice. The mix was prepared from fresh milk, sweet cream, and dry skim milk.

In all other experiments a fat content of 10 and 14 per cent was chosen to determine the difference in basic viscosity between a high fat and a low fat ice cream mix, using Whitaker's apparatus and a hand emulsifier to obtain the basic viscosity.

The composition of the ice cream mixes were as follows: No. 1, 10 per cent milk fat, 10 per cent milk solids-not-fat, 15 per cent sugar, and 0.4 per cent gelatin; No. 2, 14 per cent milk fat, 10 per cent milk solids-not-fat, 15 per cent sugar, and 0.4 per cent gelatin. The ice cream mixes were pasteurized at 68.3° C. (155° F.) for 30 minutes and homogenized at 2500 and 500 pounds pressure per square inch at the pasteurizing temperature in a Manton-Gaulin homogenizer of a 60 gallon per hour capacity. They were then cooled on a surface tubular cooler to 4° C. and a sample was promptly taken for immediate determination of original viscosity. The

ice cream mixes after being cooled were permitted to age at 4° C. for 4 and 24 hours, after which time tests were made for apparent viscosity and basic viscosity as obtained by the Whitaker device and the hand emulsifier. The viscosities were determined between 4.2–5.4° C. in a room at 4° C. with a MacMichael Viscometer using No. 26 and No. 30 wires, and the disc bob. These wires were calibrated against oils standardized by the Bureau of Standards.

At the time that the viscosity was measured a 1 ml. sample of the ice cream mix was taken and diluted with 99 ml. of distilled water for microscopic examination of fat globules and fat clumps. All of the fat globules and fat clumps were counted and measured in the field or fields until 100 fat globules were measured. This method not only gave the average size of fat clumps and fat globules but the relative ratio of fat clumps to the fat globules, in the ice cream mix. The ocular micrometer scale was standardized to make one small division equal one micron.

In breaking down the apparent viscosity with the Whitaker device (2) the recommended time of twenty minutes was used. The hand emulsifier was operated at an average of 78 full strokes per minute. All basic viscosities were read immediately after breaking down the apparent viscosity.

#### EXPERIMENTAL RESULTS

It was essential to standardize the procedure with the hand emulsifier so that results could be easily duplicated. The number of homogenizations required to bring a mix to a constant basic value, the speed of homogenization, and the constancy of data secured with different emulsifiers must be known.

Two emulsifiers were used for comparison. Emulsifier No. 1 was a new instrument while emulsifier No. 2 had light service for two years. Emulsifier No. 1 was used throughout the entire investigation.

The data, table 1, show that emulsifying the ice cream mix twice was

TABLE 1

*Basic viscosity of ice cream mixes at 4° C. as affected by the number of times emulsified*

Mix	Number of times emulsified	Viscosity in centipoises		
		0 hrs.	4 hrs.	24 hrs.
1 . . . . .	0	18	53	51
1 . . . . .	2	...	24	27
1 . . . . .	3	...	...	23*
2 . . . . .	0	36	136	271*
2 . . . . .	2	...	45	41
2 . . . . .	3	...	45	48
2 . . . . .	4	...	...	50

\* Temperatures were one degree too high.

TABLE 2  
*Relation of size and number of fat globules and clumps to the number of times the mix is emulsified*

as efficient as three or four times in breaking the apparent viscosity down to a minimum. This is substantiated by the data, table 2, which show that the average size of fat globules and fat clumps and their relative numbers were not materially affected by the number of times the mix was emulsified. The hand emulsifier hardly affected the size of the fat globules. It should be pointed out that the viscosities of the two mixes, table 1, aged 0 hours which may properly be termed "original viscosity" represent both the absolute basic values which at that moment are exactly the same as the apparent viscosity. Then too, the viscosities of the mixes are apparent when not emulsified after 4 and 24 hours aging.

The data in tables 1 and 2 show that when the size of the fat clump was small the number of clumps present in the mix did not greatly affect the viscosity as compared with the effect of individual globules.

The effect of two extreme emulsifying speeds on the basic viscosity was determined. Mixes were emulsified at 36 full strokes per minute and at 120 full strokes per minute to compare with the usual speed of 78 strokes.

TABLE 3  
*The effect of the rate of emulsifying on the basic viscosity of the mix*

Mix	Strokes per minute	Viscosity in centipoises	
		Period aged	
		0 hrs.	24 hrs.
1	0	14*	80*
1	36	..	26
1	78	..	24
1	120	..	23
2	0	37*	445*
2	36	..	61
2	78	..	56
2	120	..	54

\* Temperature 2.5 to 3.7° C. Other temperatures were 4° C.

The data, table 3, show that if the mix was emulsified at the rate of 120 full strokes per minute the basic viscosity obtained was slightly lower than that obtained by emulsifying at the rate of 36 strokes per minute. The usual speed of 78 strokes gave the same results as the very high speed and was adopted throughout this study.

Since Dahlberg (9) first showed that gelatin gel is reformed in ice cream after whipping and Dahlberg, Carpenter, and Hening (2) found this regelation to be practically completed in 10 hours it is evident that basic viscosity is temporary. It was necessary to know how quickly the reading must be made after the mix had been broken down to basic viscosity. Figure 1 shows that the basic viscosity as obtained by the hand emulsifier increased as a linear function of time for the first 30 minutes. Therefore it is advisable to determine the basic viscosity within as short

a time as possible after obtaining it. The longest time that may be permitted between the time the apparent viscosity is broken down and the sample tested for basic viscosity is five minutes. This time lag does not introduce a serious error.

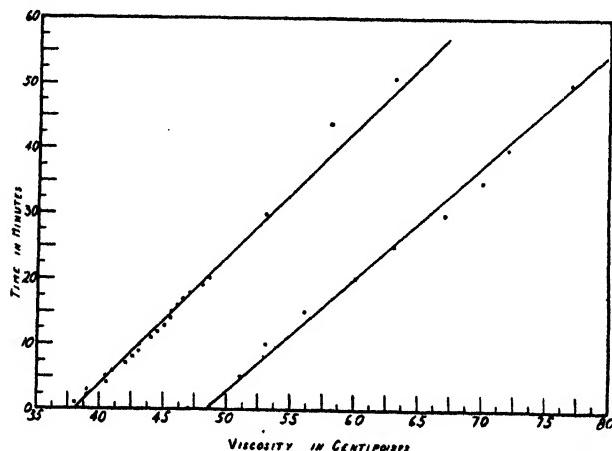


FIG. 1. The basic viscosity of two different ice cream mixes as affected by aging after hand emulsification.

It was essential to know if the results could be duplicated by another emulsifier of the same make. The apparent viscosity was broken down by two emulsifiers, one being new while the other was two years old. The data, table 4, show that the basic viscosities obtained by different emulsifiers of the same make were duplicated within reasonable experimental error.

TABLE 4  
*The results secured with two hand emulsifiers of the same make*

Mix	Emulsifiers	Viscosity in centipoises		
		Period aged		
		0 hrs.	4 hrs.	24 hrs.
1 .....	1 .....	14*	35	80*
		.....	16	24*
1 .....	2 .....	.....	18	21*
		37*	195	445*
2 .....	1 .....	.....	37	56*
		.....	40	59*

\* Temperature range between 2.4-3.5° C. instead of 4.2-5.4° C.

The hand emulsifier was then compared with the Whitaker apparatus (3) for breaking down apparent viscosity to a constant basic value. The mix was emulsified twice and immediately tested for basic viscosity. The Whitaker device was used according to his recommended procedure.

The data, table 5, clearly show that the hand emulsifier reduced mixes to lower basic values than the Whitaker device.

TABLE 5

*The comparison of basic viscosities of ice cream mixes as obtained by the hand emulsifier and the Whitaker device*

Mix	Type of agitation	Viscosity in centipoises		
		0 hrs.	4 hrs.	24 hrs.
1		18	53	151
1	Whitaker			47
1	Emulsifier		24.5	27
2		36	13.6	271
2	Whitaker			73
2	Emulsifier		45	41
2B*		37	195	445
2B	Whitaker		76.0	
2B	Emulsifier		37	

\* Mix 2B is also a 14 per cent milk fat mix.

According to Hening (4) the difference should be due to the fact that the hand emulsifier is more efficient than the Whitaker device in breaking down the fat clumps. Both methods of agitation are supposed to be vigorous enough to break down the gel structure formed by gelatin in an ice cream mix. To see if the assumption proposed by Hening is true, the average size of the fat globules and clumps as well as the number of fat clumps per 100 fat globules were determined. The data, table 6, show that the Whitaker device was as efficient as the hand emulsifier in breaking up the larger size fat clumps. Other data, table 2, indicate that the change in basic viscosity was independent of the size of the fat clumps when the fat clumps were small. Therefore the higher basic viscosity obtained by the Whitaker device was not due to the size of the fat clumps. The increase is also not due to incorporation of air because extreme precautions were taken to prevent the incorporation of air in the mix by the Whitaker device. Therefore, a test was set up to see if both methods of agitation break down the gel structure completely.

Two samples were used in this test. Sample I consisted of skimmilk with 0.8 per cent gelatin. Sample 2 was a standard mix of 12 per cent milk fat, 10 per cent milk solids-not-fat, 15 per cent sugar and 0.4 per cent gelatin. Both samples were treated as previously described but in addition to breaking down the apparent viscosity with the hand emulsifier and the Whitaker device, the large power operated homogenizer was used with a two stage valve set at 3500 and 500 pounds pressure per square inch. The homogenizer was precooled with the ice water before the tests were made.

The temperature for reading the viscosity ranged between 5.5° C. and

TABLE 6  
*The average size of fat globules and fat clumps obtained by the Whitaker device and the hand emulsifier and the number of fat clumps per 1/10 fat globules*

Mix	Type of agitation	Size of fat globules and fat clumps in microns						Number of fat clumps to 100 fat globules	
		Average size of fat globules		Average size of fat clumps		Period aged			
		0 hrs.	4 hrs.	24 hrs.	0 hrs.	4 hrs.	24 hrs.		
1	Whitaker	.79	.86	.76	.75 × 1.45	.60 × 1.25	.50 × 1.01	9	
	Emulsifier	"	"	.97	"	"	.61 × 1.29		
2	Whitaker	.81	.82	.75	1.0 × 1.73	.90 × 1.61	.73 × 1.28	50	
	Emulsifier	"	"	.79	"	"	.77 × 1.43		
2B*	Whitaker	1.12	1.19	1.19	1.6 × 2.63	1.05 × 2.31	1.12 × 1.71	38	
	Emulsifier	"	"	"	1.25	"	.93 × 1.58		
2B	Whitaker	"	"	"	1.12	"	"	28	
	Emulsifier	"	"	"	"	"	"		
								23	

\* Mix 2B is also a 14 per cent milk fat mix.

7.2° C. After the basic viscosity was read a sample was taken for each method and permitted to stand for 24 hours to determine the ability of the sample to regel.

TABLE 7

*The basic viscosity of ice cream mix and of skimmilk containing gelatin as secured by several methods and the ability to regel*

Method of obtaining basic viscosity	Skimmilk with gelatin	Ice cream mix	Viscosity of skim- milk 24 hours after being reduced to basic
Original . . . . .	10	20.5	.....
4 hours of aging (apparent) . . . . .	.....	122	.....
Homogenized 2-stage, 3500-500 . . . . .	10	27	150
Hand emulsifier . . . . .	17	34	285
Whitaker device . . . . .	34	42	245
24 hours of aging (apparent) . . . . .	560	192	.....
Homogenized 2-stage, 3500-500 . . . . .	10	24	105
Hand emulsifier . . . . .	19	43	177.5
Whitaker device . . . . .	35	53	185

The results, table 7, indicate that the hand emulsifier was more efficient in breaking down the gel structure than the Whitaker device but that neither of them broke it down as completely as did the power operated homogenizer. The power homogenizer reduced aged solutions of gelatin in skimmilk back to the original viscosity before aging. None of the methods were capable of destroying the re-gelatinizing power of the gelatin.

#### DISCUSSION

The desirability of a simple inexpensive method to obtain basic viscosity of ice cream mixes is obvious. This study was undertaken to learn if the hand emulsifier is more simple, efficient, and practical than the Whitaker device in breaking down apparent viscosity to basic viscosity. The hand emulsifier may be much more readily assembled, cleaned and operated than the Whitaker device. It is also much more economical to purchase and operate.

The devices of Dahlberg and of Whitaker reduced Leighton's time of agitation and greatly reduced the size of sample. The hand emulsifier further reduced the time. Allowing two minutes for making the basic viscosity test the method used by Leighton would require 62 minutes, the Whitaker method 22 minutes and the hand emulsifier 5 minutes to break down apparent viscosity to basic viscosity and run the test.

Although the hand emulsifier is more efficient in breaking down the apparent viscosity to basic viscosity, it does not seem to be more efficient in decreasing the size of the fat clumps. It did reduce gelatin gels more completely to a minimum basic viscosity.

#### CONCLUSIONS

It is concluded that the hand emulsifier is simpler, more practical and more efficient than the agitation methods of reducing apparent viscosity to basic viscosity.

The difference between the minimum viscosity obtained by the hand emulsifier and the Whitaker device is not due to the size of fat clumps and fat globules, but due to the fact that the hand emulsifier is more efficient in breaking down the gel structure.

The results indicate that even though the gel structure was broken down completely, as was done by the power operated homogenizer, the gelatin used in this experiment had the power to regel without change in temperature of storage.

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# EFFECT OF VARIOUS BACTERIA ON FLAVOR OF CHEDDAR CHEESE MADE FROM PASTEURIZED MILK\*

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Adequate pasteurization of milk for cheddar cheese has two advantages. It insures destruction of pathogens that may have gained entrance to the milk up to the time of heating and, accordingly, is of public health significance. Also, it tends to control various objectionable fermentations when the causative organisms enter the milk preceding the pasteurization.

Experience has shown, however, that cheddar cheese made from pasteurized milk does not develop the full, characteristic flavor normally found in raw milk cheese of fine quality. It appears that during pasteurization a change occurs which interferes with development of the typical cheese flavor and also materially increases the time necessary for ripening. Whether this change is due to partial destruction of the natural bacterial flora of the milk, to action on enzymes, or to both, is not known. If the increase in time required for ripening of the cheese is due to partial destruction of the natural flora of milk by pasteurization, addition of cultures of desirable bacteria should aid in overcoming the difficulty.

The work herein reported deals with the effect of various bacteria of the genus *Micrococcus* and the genus *Propionibacterium* on flavor of cheddar cheese made from pasteurized milk. These genera were selected because of the frequency with which organisms belonging to them have been found in raw milk cheese (2, 3, 5, 7).

## METHODS

### *Manufacture and ripening of cheese*

The cheese were made in the experimental vat designed by Lane (9). This consists of five small compartments surrounded by a common water jacket, and with it the five cheese in a series could be made with approximately identical procedures. Each cheese weighed about 2.5 pounds.

The milk used came from various herds and contained approximately 4 per cent fat. All the milk was pasteurized at 61.7° C. (143° F.) for 30 minutes, so that it would have met any public health requirement, and then cooled.

In making a series of cheese, 25 pounds of the thoroughly mixed, pasteurized milk was weighed into each compartment and heated to 30° C. (86° F.); 2.5 per cent of the usual culture was then added. One lot of milk had no further inoculation and was intended as a control, while each

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of the other four was inoculated with an organism to be tested. The milk was usually ripened 30 to 45 minutes to obtain an acidity of 0.18 to 0.20 per cent, and commercial rennet was added at the rate of 3 ounces per 1,000 pounds of milk.

After 30 to 40 minutes the curd was cut with  $\frac{1}{8}$ -inch knives and was not disturbed for 8 to 10 minutes, after which heating was begun. Over a period of 40 to 60 minutes the curd was heated slowly to  $40^{\circ}$  C. ( $104^{\circ}$  F.) and then cooked until the acidity of the whey reached 0.16 to 0.18 per cent. The curd was milled at a whey acidity of not less than 0.45 per cent. About 30 minutes after milling, salt was added at the rate of 3 per cent of the estimated weight of curd. The salt dissolved in 30 to 40 minutes, and the curd in each vat was then placed in a hoop. The cheese were pressed for about 1 hour, removed for dressing and then replaced in the press for an additional 16 hours. They were ripened at  $4.4^{\circ}$  to  $10^{\circ}$  C. ( $40^{\circ}$  to  $50^{\circ}$  F.).

#### *Examination and scoring of cheese*

The cheese were examined for flavor by two or more judges after approximately 1, 2 and 3 months of ripening. Samples were obtained with a small trier, and the trier holes were filled at once with paraffin. The cheese in a series, with the identities concealed, were ranked from 1 to 5 on the basis of the development of cheddar flavor. In tabulating the results the various cheese were recorded as being better or poorer than the control of the series.

#### RESULTS OBTAINED

##### *Effect of various micrococci on flavor of cheese*

The effect of micrococci was studied with 34 cultures which represented various types of micrococci isolated from cheddar cheese by Higdon (7). Twenty-three series of cheese were made; except in a very few instances, each included a control and four cheese made with different test organisms. The cultures of micrococci were prepared by inoculating into sterile milk and incubating 3 days at  $21^{\circ}$  C. ( $69.8^{\circ}$  F.); 0.05 per cent of such a culture was added to the cheese milk. Each culture was used in one to five trials, depending on its effect on cheese flavor.

The cultures of micrococci were divided into three groups according to their effects on cheese flavor: Those having an undesirable effect, those having no definite effect and those having a desirable effect. The allocation of a culture to a group was based not only on whether the culture gave cheese which was placed above or below the control but also on the extent to which the flavor differed.

##### *Cultures having an undesirable effect*

Seven of the 34 cultures were considered to have an undesirable effect on cheese flavor. The data obtained with them are summarized in table 1.

TABLE 1

*General effect of various micrococci on flavor of cheddar cheese from pasteurized milk  
(Cultures having an undesirable effect)*

Culture no.	No. of trials	Results after						Summary of results	
		1 month		2 months		3 months			
		No. of trials better than control	No. of trials poorer than control	No. of trials better than control	No. of trials poorer than control	No. of trials better than control	No. of trials poorer than control	No. of trials better than control	No. of trials poorer than control
1	1	0	1	0	1	0	1	0	3
2	3	0	3	1	2	1	2	2	7
3	4	2	2	0	4	2	2	4	8
4	1	0	1	1	0	0	1	1	2
5	1	0	1	0	1	0	1	0	3
6	4	0	4	2	2	1	3	3	9
7	4	2	2	1	3	2	2	5	7

The cheese made with the seven cultures commonly developed bitter or unnatural flavors. In some cases these flavors appeared rather early in the ripening process, while in others they did not develop until relatively late. Some cultures produced bitterness after 1 month but, as the cheese aged, the bitter flavor had a tendency to disappear.

*Cultures having no definite effect*

Of the 34 cultures, 14 had no definite effect on cheese flavor. Table 2 summarizes the data on them.

TABLE 2

*General effect of various micrococci on flavor of cheddar cheese from pasteurized milk  
(Cultures having no definite effect)*

Culture no.	No. of trials	Results after						Summary of results	
		1 month		2 months		3 months			
		No. of trials better than control	No. of trials poorer than control	No. of trials better than control	No. of trials poorer than control	No. of trials better than control	No. of trials poorer than control	No. of trials better than control	No. of trials poorer than control
8	4	1	3	2	2	3	1	6	6
9	2	2	0	0	2	1	1	3	3
10	2	1	1	0	2	2	0	3	3
11	2	2	0	1	1	0	2	3	3
12	2	2	0	0	2	1	1	3	3
13	2	1	1	2	0	0	2	3	3
14	2	0	2	1	1	2	0	3	3
15	2	0	2	1	1	2	0	3	3
16	2	0	2	2	0	1	1	3	3
17	2	2	0	1	1	1	1	4	2
18	2	2	0	1	1	1	1	4	2
19	1	1	0	0	1	1	0	2	1
20	1	1	0	0	1	1	0	2	1
21	5	3	2	3	2	2	3	8	7

A number of the cultures produced cheese that after 1 month were better than the controls, but after 3 months bitterness and other off flavors had developed. Other cultures produced undesirable flavors after 1 month, but after 3 months the cheese had improved.

### *Cultures having a desirable effect*

Thirteen of the 34 cultures generally improved the flavor of cheese made with them. A summary of the data is given in table 3.

TABLE 3

*General effect of various micrococci on flavor of cheddar cheese from pasteurized milk  
(Cultures having a desirable effect)*

Culture no.	No. of trials	Results after						Summary of results	
		1 month		2 months		3 months			
		No. of trials better than control	No. of trials poorer than control	No. of trials better than control	No. of trials poorer than control	No. of trials better than control	No. of trials poorer than control	No. of trials better than control	No. of trials poorer than control
22	2	2	0	0	2	2	0	4	2
23	2	1	1	1	1	2	0	4	2
24	2	0	2	2	0	2	0	4	2
25	2	0	2	2	0	2	0	4	2
26	2	2	0	0	2	2	0	4	2
27	2	2	0	1	1	2	0	5	1
28	2	1	1	2	0	2	0	5	1
29	4	3	1	3	1	2	2	8	4
30	3	3	0	1	2	2	1	6	3
31	4	4	0	3	1	3	1	10	2
32	5	4	1	3	2	4	1	11	4
33	4	2	2	2	2	3	1	7	5
34	2	1	1	2	0	2	0	5	1

In general, the cultures produced flavors more or less characteristic of well ripened cheddar cheese. Some of them gave good flavors in the cheese regularly throughout the ripening period. A few, however, produced undesirable flavors after 1 month, but the cheese had improved after 3 months and were better than the controls.

### *Comparison of numbers of micrococci at beginning and end of ripening*

With each cheese prepared by adding micrococci to pasteurized milk, a count of the micrococci was made on the fresh curd and on the cheese 3 months old. One gram of curd or cheese was ground with 9 ml. of 2 per cent aqueous sodium citrate and 0.1 ml. portions of various dilutions were spread over the surface of solidified beef infusion agar (in plates) with a sterile bent glass rod; incubation was at 21° C. (69.8° F.) for 5 to 7 days. On the surface of the agar *Micrococcus* colonies could be identified with considerable accuracy. Although some of the micrococci may have survived

pasteurization or come from the equipment, air, etc., the colonies on the plates probably represented primarily the cultures added to the milk since the numbers of micrococci present in the control cheese were regularly very low.

Counts of the micrococci on the fresh curd varied from 100,000 to 57,000,000 per gram, while counts on the ripened cheese varied from 10,000 to 17,000,000 per gram. In general, the numbers of micrococci were lower at the end of the ripening period than at the beginning, but a few cultures gave a definite increase in some of the trials. In instances where counts were lower at the end of the ripening period than originally, there may have been a conspicuous increase and then a decrease in the numbers.

The general changes in numbers of micrococci during ripening were not related to the development of characteristic cheddar flavor. Cultures which had desirable effects on the cheese were lower in numbers at the end of the ripening than originally as often as those which gave undesirable results.

#### *Variation in cultures belonging to the same species*

The 34 cultures employed represented as varied a selection as possible of micrococci isolated from cheddar cheese. They were grouped by Higdon (7) into 23 species of which only 12 could be identified. Cultures which were classified as belonging to the same species did not have comparable effects on the flavor of cheese, which suggests that if micrococci are employed in the manufacture of cheddar cheese they should be selected on a strain basis rather than a species basis.

#### *Effect of certain propionic acid bacteria on flavor of cheese*

The effect of propionic acid bacteria was studied with seven cultures, four of which came from cheddar cheese and three from Swiss-type cheese. The general procedure was the same as with the micrococci. The cultures were prepared by inoculating the organism into tomato broth and incubating 3 days at 30° C. (86° F.); 0.05 per cent of culture was added to the cheese milk. The data are summarized in table 4.

Some of the cultures of propionic acid bacteria produced a desirable flavor in the cheese throughout the ripening period. Others produced an undesirable flavor after 1 month but developed a desirable flavor after 3 months or produced a desirable flavor after 1 month and then, as the cheese ripened, bitterness and other off flavors appeared.

Two additional series of cheese were manufactured in which very large numbers of cultures 1, 2, 3 or 4 were added to the milk. The large numbers of these organisms in cheese produced a desirable effect. Even with cultures 2 or 3, which did not improve the flavor of the cheese when used in small numbers (table 4), the large numbers were beneficial. All the cheese made with the large numbers of organisms had a very desirable flavor after ripen-

ing 3 months. Some of the cheese had a distinct sweet flavor, somewhat similar to that characteristic of Swiss cheese, but no eyes developed.

In Swiss-type cheese the growth of the propionic acid organisms results in the formation of propionates which have a desirable effect on the flavor (1). Since these organisms under certain conditions improved the flavor of cheddar cheese, the effect of calcium propionate on the flavor of cheddar cheese was investigated by adding small amounts to cheddar cheese being made into process cheese. In general, the effects were desirable. The

TABLE 4  
*General effect of certain propionic acid bacteria on flavor of cheddar cheese from pasteurized milk*

Culture no.	No. of trials	Results after						Summary of results	
		1 month		2 months		3 months			
		No. of trials better than control	No. of trials poorer than control	No. of trials better than control	No. of trials poorer than control	No. of trials better than control	No. of trials poorer than control	No. of trials better than control	No. of trials poorer than control
1	5	1	2	2	1	3	0	6	3
2	3	1	2	1	2	3	0	5	4
3	3	2	1	1	2	1	2	4	5
4	3	2	1	2	1	3	0	7	2
5	1	1	0	1	0	0	1	2	1
6	2	2	0	2	0	2	0	6	0
7	1	1	0	1	0	0	1	2	1

amounts which were most beneficial varied with the original cheese. With the larger amounts used the flavor was too sweet and suggestive of Swiss-type cheese.

#### *Comparison of numbers of propionic acid bacteria at beginning and end of ripening*

Most of the cheese manufactured with addition of propionic acid bacteria to the pasteurized milk were examined for the numbers of these organisms at the beginning and end of the ripening period. The counts were obtained by grinding 1 gram with 2 per cent aqueous sodium citrate and preparing shake cultures in sodium lactate agar (2). Colonies showing the usual characteristics were counted as propionic acid organisms.

The counts of propionic acid bacteria on the fresh curd varied from 1,000,000 to 17,000,000 per gram, while the counts on the ripened cheese varied from 100,000 to 9,000,000 per gram. In practically all cases the numbers of the organisms were lower at the end of the ripening period than at the beginning.

#### **DISCUSSION OF RESULTS**

The data suggest that certain strains of micrococci may be useful in making cheddar cheese from pasteurized milk. These results are in agree-

ment with studies of Lane (8) and of Hansen (4), both of whom reported that an unidentified *Micrococcus* improved the flavor of pasteurized milk cheese. The products produced by micrococci, as reported by Suzuki *et al.* (10) and by Hart *et al.* (6), include compounds which presumably might have a desirable effect on the flavor of cheese.

If a *Micrococcus* is to be employed in making cheddar cheese from pasteurized milk, it should be selected on a strain rather than a species basis because of the differences between cultures apparently belonging to the same species. Such a selection is used with the propionic acid cultures employed in the manufacture of Swiss-type cheese, with the citric acid fermenting streptococci and *Streptococcus lactis* used in developing butter cultures, with the molds used in making blue cheeses and in various other fields of dairy ing.

The results also indicate that certain strains of propionic acid bacteria may be useful in making cheddar cheese from pasteurized milk. If such an organism is to be employed, careful selection of the strain apparently is advisable. Since the organisms normally convert some of the lactic acid to propionic and acetic acids and carbon dioxide, propionates undoubtedly are formed in the cheese, some of which have a sweet flavor. Propionates are largely responsible for the sweet flavor of Swiss-type cheese and in small amounts they may be desirable in cheddar cheese; in this connection the results obtained when calcium propionate was added to cheddar cheese being made into process cheese are suggestive.

It appears that the flavor of cheddar cheese can be influenced by a variety of bacteria, including various streptococci, lactobacilli, micrococci, propionic acid bacteria and probably other types. This general relationship suggests that a desirable flavor in cheddar cheese may not always be brought about through the action of the same combination of organisms but rather that one combination may be responsible in one lot of cheese and another combination in another lot. The great variation in flavor of cheddar cheese of good quality is in agreement with such a relationship.

Because of the public health angle and the wide application of pasteurization in the dairy industry, the manufacture of cheddar cheese from pasteurized milk will probably be extended. The general results indicate that there may be a distinct advantage in inoculating the heated milk with organisms other than the usual cheese cultures. The cheese cultures alone do not establish conditions which result in a ripening comparable to the ripening of good raw milk cheese. It may be necessary to use a combination of organisms if the pasteurized milk cheese is to be of a quality comparable to that of the best raw milk cheese. There remains the possibility that addition of one or more enzymes to the milk will be more practical than use of a combination of bacteria.

## SUMMARY

1. Thirty-four *Micrococcus* cultures, originally isolated from cheddar cheese, had the following effects on the flavor when inoculated into pasteurized milk made into cheddar cheese: Seven had undesirable effects, producing bitter and unnatural flavors; fourteen had no definite effects; thirteen had desirable effects.
2. Strains of micrococci apparently belonging to the same species differed in their effects on the flavor.
3. When used in relatively small numbers, certain strains of propionic acid bacteria improved the flavor of pasteurized milk cheddar cheese, while others had no definite effects; when used in large numbers all the strains improved the flavor, and some of them produced a distinct sweet flavor somewhat resembling that of Swiss cheese.
4. The results suggest the possibility of improving the flavor of pasteurized milk cheddar cheese by the addition to the milk of various organisms other than the usual cheese cultures.

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## A STUDY OF NICKING IN JERSEY CATTLE

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"Nicking" has been the subject of considerable discussion among breeders for many years. Generally speaking, this term is applied to those cases where the progeny from certain matings appear superior to those from other matings of the same animal or families. Webster defines nicking as follows: "In stock breeding, to combine well."

Breeders are constantly seeking to improve both type and production. In doing so they try different outeross matings, linebred matings, and inbred matings, hoping to purify or intensify desirable qualities. When one of these types of matings appears superior to the others it is said to "nick."

This factor of superior progeny has been noticed in other types of livestock. In Herefords it is a recognized fact that when Anxiety IV was mated to daughters of North Pole, the resulting progeny were much superior to those of other matings. In Standard Bred horses the crossing of the descendants of Peter the Great with those of Axworthy has given such superior, uniform progeny that it is known as the Golden Cross.

Hervey and Heizer (1) have shown in comparing daughter-dam groups of the various matings of three Guernsey sires that certain crosses resulted in significant differences in production. They attribute these differences to nicking. Fohrman and Graves (2) made a study of the daughters of 51 Ayrshire sires. In one case they found that the daughters of Sire 36 out of dams by Sire 11 averaged higher in production than did those daughters of Sire 36 out of dams by miscellaneous sires. In direct contrast to this an editorial in Hoard's Dairyman (3), in discussing nicking, states that the Pennsylvania State College has records of 100 proved bulls in which it finds that those proved to be prepotent in one herd were prepotent in other herds and vice versa. These conflicting opinions raise the question: "Will a bull proved to transmit high producing capacity in one herd be equally successful in another herd where a different family blood line exists?"

### SOURCE OF DATA AND TREATMENT

In an attempt to throw more light on this problem a study was made in cooperation with the American Jersey Cattle Club. Register of Merit

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records were studied to see if there might be a difference in the production-capacity of various matings. Jersey bulls having the largest number of tested progeny out of tested dams were selected for analysis. It was preferable that each bull have two or more groups of daughters, each group being out of dams by one sire. It was necessary for the bull to have at least six tested daughters out of dams by one sire for each group. These requirements greatly reduced the number of bulls available for study.

Two types of comparative study were made. In the first, the groupings consisted of tested daughters out of tested dams. In the second, the groupings consisted of tested daughters only because there were not enough tested dams available. As previously stated, in grouping the animals the data include not less than six daughter-dam pairs, or six daughters in a group.

This was the smallest number that it was felt advisable to use. Davidson (4) has concluded that the average production and variability of the first fifteen daughters of a sire is representative of any larger number of tested daughters and furthermore, that on the average, six is the smallest number of first tested daughters whose average production closely approximates that of the first fifteen. His correlation of .91 between the first six and the first fifteen must be discounted somewhat however, since the first fifteen also include the first six, which would make a partial correlating of a thing with itself.

Any number of less than six daughters by this method of grouping was placed in the miscellaneous classification. If a cow had more than one daughter in a group her records were repeated as many times as she had daughters in that group.

All records were converted to a three time a day, 365 day, mature basis. Conversion factors were those used by the American Jersey Cattle Club (5). All records of less than 270 days were discarded as being abnormal. Butterfat alone was used in making these comparisons. Graves (6), Roberts (7), and Wilson (8), have concluded that milk and butterfat percentage are inherited separately with total butterfat the result of the product of these two. Thus, total butterfat would be the best single criterion to use in making these comparisons.

#### RESULTS

In tables 1, 2, and 4, the mean or average production of each of the groups is listed together with its probable error (P.E.) and is followed by the mean or average deviation.

Table 1 presents a summary of seventeen sires where daughter-dam comparisons were available. In examining this table it will be noticed that in most of the cases there are no apparent significant differences.

Sophie 19th's Victor seemed constant for a certain level of production

TABLE 1  
*Daughter-dam comparisons by sire groups*

Sire of dams	Pairs	Mean and probable error		Mean deviation	
		Dams	Daughters	Dams	Daughters
<b>A. Sophie 19th's Victor 171861</b>					
All sires .....	52	725 ± 10	700 ± 8	103	86
Pogis 99th of Hood Farm .....	15	746 ± 15	692 ± 12	88	67
Sophie 19th's Tormentor .....	10	687 ± 13	708 ± 22	63	101
Miscellaneous .....	27	728 ± 16	702 ± 12	122	90
<b>B. Volunteer's Dreaming Sam 261643</b>					
All sires .....	27	576 ± 8	584 ± 9	61	71
Bowlina's Sultan .....	7	594 ± 10	596 ± 22	40	88
Miscellaneous .....	20	569 ± 10	580 ± 10	65	65
<b>C. Thistle's Fairy Boy 268501</b>					
All sires .....	40	515 ± 8	465 ± 5	72	48
Sally's Fairy Boy .....	21	550 ± 11	474 ± 7	76	48
Roberta's Golden Tycoon .....	11	459 ± 6	448 ± 8	29	39
Miscellaneous .....	8	498 ± 16	466 ± 14	67	58
<b>D. Fauvie Maid's You'll Do 225661</b>					
All sires .....	36	632 ± 8	656 ± 7	72	60
Lotus Gold Medal .....	11	623 ± 10	659 ± 12	48	59
Sprite's Baron .....	10	674 ± 17	693 ± 13	80	63
Miscellaneous .....	15	610 ± 12	630 ± 8	69	44
<b>E. Sybil's Successor 258883</b>					
All sires .....	37	597 ± 10	600 ± 13	89	114
Sybil's Gamboge .....	10	558 ± 16	624 ± 25	77	117
Miscellaneous .....	27	609 ± 12	591 ± 15	91	112
<b>F. Benedictine Ruler 215113</b>					
All sires .....	37	625 ± 10	585 ± 9	94	84
Jolly's Cowship of P. H. ....	23	656 ± 15	600 ± 13	108	91
Miscellaneous .....	14	575 ± 11	561 ± 12	64	67
<b>G. Spermfield Owl's Progress 163331</b>					
All sires .....	77	709 ± 5	686 ± 5	68	63
Sibley's Choice .....	14	700 ± 11	689 ± 10	61	57
Sibley's Interested Prince .....	9	711 ± 4	679 ± 8	18	34
Sibley's Interested Owl .....	6	721 ± 22	704 ± 19	81	68
Sue B's Omega Choice .....	6	598 ± 0	715 ± 21		78
Miscellaneous .....	42	725 ± 7	679 ± 7	70	68
<b>H. Cedarine Golden Sultan 253221</b>					
All sires .....	24	529 ± 9	541 ± 6	66	49
You'll Do's Volunteer .....	12	501 ± 16	570 ± 8	83	42
Imp. Nobly Born .....	6	526 ± 12	545 ± 9	44	32
Miscellaneous .....	6	587 ± 11	477 ± 18	40	65
<b>I. Combination's Pretty Lad 205788</b>					
All sires .....	66	536 ± 7	506 ± 6	87	74
Fairy Glen's Raleigh of S. V. ....	20	517 ± 10	470 ± 8	66	55
Topsy's Sensational Lad .....	10	601 ± 21	551 ± 13	107	59
Miscellaneous .....	36	528 ± 9	514 ± 9	79	80

TABLE 1.—(Continued)

Sire of dams	Pairs	Mean and probable error		Mean deviation	
		Dams	Daughters	Dams	Daughters
J. Design's Mighty Sovereign 336549					
All sires .....	18	664 ± 8	640 ± 9	47	54
Fauvie Maid's You'll Do .....	10	636 ± 8	639 ± 13	39	62
Miscellaneous .....	8	698 ± 8	641 ± 11	33	45
K. Owl-Interest Mercury 217413					
All sires .....	24	583 ± 10	577 ± 12	72	88
Raleigh's LaRilla Lad .....	6	604 ± 29	668 ± 19	107	68
Sultana's Virginia Lad .....	6	571 ± 14	554 ± 20	52	71
Miscellaneous .....	12	578 ± 12	542 ± 26	64	91
L. St. Mawes Lad 130501					
All sires .....	22	756 ± 14	813 ± 19	98	129
Rinda Lad of S. B. ....	7	754 ± 21	887 ± 30	82	116
Miscellaneous .....	15	757 ± 18	779 ± 21	105	119
M. Rosaire's Olga Lad 87498					
All sires .....	46	691 ± 8	682 ± 10	84	101
St. Mawes .....	31	689 ± 11	692 ± 12	90	103
Miscellaneous .....	15	693 ± 12	661 ± 17	70	100
N. Silver Chimes of S. B. 96021					
All sires .....	37	642 ± 8	664 ± 11	72	92
Rosaire's Olga Lad .....	14	678 ± 7	719 ± 19	40	103
Sampson Exile .....	15	624 ± 13	620 ± 13	73	73
Miscellaneous .....	8	615 ± 18	652 ± 21	77	90
O. Rinda Lad of S. B. 89518					
All sires .....	35	614 ± 6	722 ± 12	49	102
Golden Fern's Noble Jr. ....	7	641 ± 23	744 ± 27	90	106
Mistletoe Pogis .....	11	596 ± 9	684 ± 23	44	113
Miscellaneous .....	17	614 ± 6	738 ± 11	37	68
P. Poppy's St. Mawes 115434					
All sires .....	25	728 ± 10	652 ± 12	73	92
Rosaire's Olga Lad .....	16	745 ± 9	620 ± 16	56	96
Miscellaneous .....	9	699 ± 21	708 ± 17	94	76
Q. You'll Do Raleigh Oxford 169586					
All sires .....	36	675 ± 9	704 ± 11	81	97
LeCros .....	23	657 ± 10	705 ± 14	71	98
Golden Fern's Pathfinder .....	7	772 ± 2	732 ± 36	9	143
Miscellaneous .....	6	631 ± 20	665 ± 8	73	28

regardless of the production of the dams to which he was mated. Sophie 19th's Victor, Sophie 19th's Tormentor, and Pogis 99th of Hood Farm are all maternal brothers, being sons of Sophie 19th of Hood Farm. The daughters of Sybil's Successor appeared to average higher when he was mated to paternal sisters of his sire, Sybil's Gamboge 3d. However, in the miscellaneous group six of his daughters out of his own paternal sisters (by Sybil's Gamboge 3d) and out of dams by Imp. Sybil's Gamboge 4th

(a full brother to Sybil's Gamboge 3d) averaged only 474 pounds. This indicated that close inbreeding did not seem desirable.

Where Spermfield Owl's Progress was mated to dams by Sue B's Omega Choice all six daughters were out of one dam who had one lower than average record. This probably accounts for the wide difference in this group. Cedarine Golden Sultan showed a large decrease in the miscellaneous group. On closer examination of this group it was found that one daughter out of a half sister to Sultan produced only 382 pounds of butterfat in one lactation while her dam averaged 695 pounds for five lactations. Another daughter had a short time record of 378 pounds in 283 days. The remaining four daughters produced about the same as those in the other groups.

The matings of Owl-Interest Mercury and St. Mawes Lad indicate that a nicking factor might be present as do those of Rosaire's Olga Lad. In the miscellaneous group, of the matings of Rosaire's Olga Lad, three inbred daughters averaged only 513 pounds while the remaining twelve averaged 698 pounds indicating that inbreeding was not favorable. Silver Chimes of S. B. showed a decided difference in the matings to dams by Rosaire's Olga Lad and Sampson Exile. The daughters of Poppy's St. Mawes out of his paternal sisters showed a decrease as compared to matings to daughters of non-related sires.

Four of the bulls presented in table 1 indicate that the differences of the production of their daughters by various matings may be accounted for by a nicking factor. A more detailed analysis of these bulls, namely, Owl-Interest Mercury, St. Mawes Lad, Silver Chimes of S. B., and Poppy's St. Mawes, is presented in table 2. Further grouping is made by listing the maternal grandsire of the dams of the daughters in each sire group and also the immediate sire in the miscellaneous group.

The sub-grouping of the daughters of Owl-Interest Mercury shows that in the miscellaneous group those out of dams by Manora's Fairy Lad increased in production slightly over their dams. Manora's Fairy Lad is of similar breeding to Raleigh's LaRilla Lad.

Where St. Mawes Lad was mated to granddaughters of Rinda Lad his daughters averaged nearly as high as where he was mated to daughters of Rinda Lad, although there was a slight decrease from the production of their dams. Further analysis of the data shows that these four dams are out of cows that averaged only 688 pounds of fat. This increase of 190 pounds (878 minus 688) is even greater than the 133 pound increase where St. Mawes Lad was mated to dams by Rinda Lad. These figures indicate that instead of a nicking factor being present it might be that the daughters of Rinda Lad were transmitting higher production to their daughters than can be justified by their own records and regardless of the sires to which they were mated.

TABLE 2  
*Detailed study of matings of four bulls with indications of nicking*

	Dams		Daughters		Mean deviation	
	No.	Mean and probable error	No.	Mean and probable error	Dams	Daughters
<b>Owl-Interest Mercury</b>						
Raleigh's LaRilla Lad .....	4	604 ± 29	6	668 ± 19	107	68
Sultana's Virginia Lad .....	3	557 ± 30	4	671 ± 20	89	58
Daisy's Prince of St. L. ....	1	699 .....	2	662 ± 18	..	37
Sultana's Virginia Lad .....	3	571 ± 14	6	554 ± 20	53	71
Bessie Bates Lad .....	2	519 ± 6	3	613 ± 25	15	63
Daisy's Prince of St. L. ....	1	623 .....	3	494 ± 8	..	21
Miscellaneous .....	10	578 ± 12	12	542 ± 26	64	91
Manora's Fairy Lad .....	3	558 ± 1	5	573 ± 21	4	70
Remainder .....	7	593 ± 23	7	520 ± 27	89	106
<b>St. Mawes Lad</b>						
Rinda Lad of S. B. ....	6	754 ± 21	7	887 ± 30	82	116
Miscellaneous .....	12	757 ± 18	15	779 ± 21	105	119
Golden Fern's Noble Jr. ....	2	854 ± 17	4	907 ± 28	50	83
George St. Mawes .....	1	862 .....	1	608 .....	..	..
St. Mawes Lad .....	1	990 .....	1	960 .....	..	..
Rinda Lad of S. B. ....	4	878 ± 15	6	866 ± 31	55	111
Remainder .....	8	677 ± 15	9	720 ± 18	66	78
<b>Silver Chimes of S. B.</b>						
Rosaire's Olga Lad .....	6	678 ± 7	14	719 ± 19	40	103
Sampson Exile .....	3	694 ± 3	10	719 ± 22	14	102
Alphena's Chief .....	2	583 ± 12	3	767 ± 37	30	95
King Koffee's Count .....	1	581 .....	1	798 .....	..	..
Sampson Exile .....	7	624 ± 13	15	620 ± 13	73	73
King Koffee's Count .....	5	648 ± 13	12	625 ± 15	69	77
Sampson Exile .....	1	476 .....	2	597 .....	..	92
Alphena's Chief .....	1	636 .....	1	607 .....	..	..
Miscellaneous .....	5	615 ± 18	8	652 ± 21	77	90
King Koffee's Count .....	2	547 ± 9	4	582 ± 22	28	64
Alphena's Chief .....	1	668 .....	2	803 .....	..	70
Silver Chimes of S. B. ....						
Rosaire's Olga Lad .....	1	819 .....	1	630 .....	..	..
Sampson Exile .....	1	579 .....	1	652 .....	..	..
<b>Poppy's St. Mawes</b>						
Rosaire's Olga Lad .....	10	745 ± 9	16	620 ± 16	56	96
St. Mawes .....	10	745 ± 9	16	620 ± 16	56	96
Miscellaneous .....	9	699 ± 21	9	708 ± 17	94	76
St. Mawes .....	4	693 ± 37	4	658 ± 12	111	37
Remainder .....	5	704 ± 24	5	748 ± 27	79	89

The data in table 2 did not make any appreciable change from that found in table 1 on the daughters of Silver Chimes of S. B. out of dams by Rosaire's Olga Lad and Sampson Exile. The  $99 \pm 23$  pound difference between these two daughter groups is the most extreme of any of the cases presented. It is the only one greater than four times its probable error which would make it have statistical significance according to Holzinger

(9). This is the best example of nicking that this study was able to show.

Inbreeding did not seem favorable for Poppy's St. Mawes. In the miscellaneous group his maternally inbred daughters (St. Mawes is his maternal grandsire) showed a decrease when compared to the daughters out of dams by non-related sires.

The possible influence of relationship matings is presented in table 3.

TABLE 3

*Daughter-dam comparison of type of mating for Poppy's St. Mawes 115434*

	Range of inbreeding (F <sub>x</sub> )	No. of pairs	Ave. prod. of dams	Ave. prod. of daughters	Increase or decrease
Own dam	.2500	1	910	679	- 231
Paternal sisters	.1356 to .1875	10	745	620	- 125
Maternal sisters	.0625 to .1250	3	621	651	+ 30
Outeross	.0000	5	704	748	+ 44

Wright's coefficient of inbreeding (10) was used to show the range of inbreeding.

In addition to the sires already presented several were studied where only daughter averages could be compared. Six of the more typical cases are presented in table 4. This phase may be of importance in a nicking study because all of the tested daughters of the sire are included. The production of the daughters of a bull are often used as a comparison when nicking is discussed and one might be justified in calling matings resulting in a higher averaging group, a case of nicking, since the production of the dams are unknown. However, the influence of the dams may be an important factor as already has been shown and for this reason too much significance should not be attached to the data in table 4.

Pogis 99th of Hood Farm seemed to mate more favorably with dams by Hood Farm Torono than he did with own paternal half sisters. Sophie 19th's Tormentor, a maternal half brother to Pogis 99th of Hood Farm appeared to mate most favorably with dams by Pogis 99th and his sire, Hood Farm Pogis 9th. The data on Sybil's Successor show that matings to dams by his sire Sybil's Gamboge 3d and to those by the full brother to Gamboge 3d, Imp. Sybil's Gamboge 4th, were not favorable but that matings to dams by his paternal grandsire, Sybil's Gamboge did seem favorable.

You'll Do's Volunteer and Masterman of Oaklands are both grandsons of Imp. Golden Fern's Noble. The linebred matings in this case appear

TABLE 4  
*Sires with daughter average comparisons*

Sire of dams	Number	Daughters	
		Mean and probable error	Mean deviation
<b>Pogis 99th of Hood Farm 94502</b>			
All sires .....	119	684 ± 5	87
Hood Farm Torono .....	48	703 ± 9	90
Hood Farm Pogis 9th .....	17	672 ± 14	86
Hood Farm Torono 20th .....	14	669 ± 10	54
Miscellaneous .....	40	672 ± 10	89
<b>Sophie 19th's Tormentor 113302</b>			
All sires .....	101	632 ± 6	83
Pogis 99th of Hood Farm .....	24	673 ± 10	71
Hood Farm Torono .....	10	624 ± 20	93
Hood Farm Pogis 9th .....	6	690 ± 9	32
Miscellaneous .....	61	612 ± 7	79
<b>Combination's Pretty Lad 205788</b>			
All sires .....	86	505 ± 5	67
Fairy Glen's Raleigh of S. V. ....	23	469 ± 7	53
Topsy's Sensational Lad .....	12	547 ± 10	53
Westgate's Royal Majesty .....	7	548 ± 17	67
Brenda's Noble Prince .....	6	488 ± 11	41
Miscellaneous .....	38	509 ± 8	76
<b>Sybil's Successor 258883</b>			
All sires .....	87	600 ± 7	95
Sybil's Gamboge .....	11	614 ± 24	117
Sybil's Gamboge 3d .....	8	550 ± 27	113
Sybil's Gamboge 4th .....	7	553 ± 20	78
Miscellaneous .....	61	610 ± 8	87
<b>You'll Do's Volunteer 238112</b>			
All sires .....	77	547 ± 5	70
Xenia's Sultan .....	20	545 ± 11	73
Masterman of Oaklands .....	8	599 ± 14	58
Miscellaneous .....	49	539 ± 6	66
<b>Sophie's Agnes' Laddie 179327</b>			
All sires .....	62	640 ± 9	107
Sophie 19th's Tormentor .....	11	651 ± 19	94
Royal Majesty of St. Cloud .....	9	666 ± 10	85
Sophie 19th's Victor .....	6	720 ± 32	117
Miscellaneous .....	36	616 ± 12	108

more favorable than the matings to daughters of Xenia's Sultan, a non-related bull. Inbreeding also seemed more favorable in the case of Sophie's Agnes' Laddie. Sophie 19th's Victor is both a maternal brother to the sire of Sophie's Agnes' Laddie and a grandson of this same bull, namely, Sophie 19th's Tormentor.

## SUMMARY AND CONCLUSIONS

In summarizing the results of this study of nicking it seems logical that there are several points to consider before one can make definite conclusions whether nicking does or does not exist among Jersey cattle. In the first place the fact that a bull has been bred to the daughters of two or three different bulls with no evidence of nicking does not necessarily mean that if this same bull is bred to the daughters of still another bull, nicking would not appear. In the second place, an apparent case of nicking may be caused by a group of dams that are transmitting higher production to their daughters than their own records would show. The matings of St. Mawes Lad indicated that the transmitting ability of the dam may be an important factor that could be overlooked in a study of this nature.

The fact that a bull may not nick as well with the daughters of one bull as he will with the daughters of another bull does not mean that this bull is a failure in the less favorable mating. This can be pointed out in the matings of Rinda Lad of S. B. When Rinda Lad was mated to dams by Golden Fern's Noble Jr. the dams averaged 641 pounds of butterfat and their daughters averaged 744 pounds. The dams by Mistletoe Pogis averaged 596 pounds and their daughters averaged 684 pounds. The mating to dams by Mistletoe Pogis did not nick as well as the mating to dams by Golden Fern's Noble Jr., but the mating to dams by Mistletoe Pogis would certainly not be called a failure.

Since close inbreeding is not a recommended practice a comparison of inbred matings to that of outeross matings is questionable for use in a study of nicking. This study has shown that inbreeding may result in increased production or it may result in a decrease when compared to other matings.

Environment is a factor of great importance that could not be considered. It could easily account for the difference in production in many of the cases. Then, too, when comparing six daughter-dam pairs to a much larger number in another group the difference may be due to a lack of sufficient data. In most cases the smaller number might be representative for the group but it would not necessarily be true in all cases.

While this study has not shown nicking to be a prevailing factor among Jersey cattle it does not justify the conclusion that nicking does not exist among Jerseys. The matings of one bull did show a pronounced difference that would be called nicking. While nicking may exist it seems quite logical that it has been used far too much as a convenient term to explain differences in production of the daughters of a bull that may be due to other causes. More daughter-dam production comparisons under similar environment are necessary to make possible a more thorough study of this subject.

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# THE ADSORPTION OF THE VITAMIN A SUPPRESSING FACTOR FROM SOYBEAN OIL<sup>1</sup>

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In previous publications (1, 2, 3, 4) it has been shown that soybeans, when fed to dairy cows, interfere with the transference of the vitamin A potency of the ration to the milk fat secreted by the cows. It has been found that the factor responsible for this action is thermo-stable; that it is distributed in both the soybean oil and the soybean oil meal produced by either the expeller or solvent processes; that prolonged extraction of the soybeans first with ethyl ether and then with ethanol failed to completely remove this factor; and, that this suppressing action is not due to the oil itself but rather to some other factor in the beans or the soybean oil.

Although much of the vitamin A suppressing factor is held by the soybean oil meal with such tenacity as to withstand prolonged extraction with fat solvents, a considerable portion is found in soybean oil. Since the oil-free meal still possesses a suppressing action, it became apparent that this effect is not due to the oil but to some substance which is partially retained by the oil meal and partially dispersed in the oil. Therefore, it seemed logical to assume that this substance which is dispersed in the oil might be removed from the oil by suitable absorbents.

## EXPERIMENTAL

The crude soybean oil used in these experiments was produced in a commercial soybean plant by the expeller process. In attempting to remove the vitamin A suppressing factor, two portions of this oil were treated with two different types of adsorbents.

As the first type of adsorbent, an activated carbon ("Nuchar") was selected. The crude soybean oil was heated to 140° C., treated with 60 grams of Nuchar per liter of oil, stirred and filtered.

For the second type of adsorbent, a synthetic sodium aluminum silicate, which has been developed by Kraybill, Brewer and Thornton, was used (5). They had found that this adsorbent possessed unusual properties for adsorbing phosphatides and associated compounds. Since the treatment of soybean oil with this adsorbent produces a clear and highly refined oil, the possibility existed that this refining process might also remove the vitamin A suppressing factor. A quantity of treated oil, which had been prepared by

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passing the crude soybean oil through a column of this adsorbent, was secured from Dr. Thornton.

In order to test the vitamin A suppressing effect of these oils, they were incorporated in the rations of a dairy cow and the effect upon the vitamin A potency of the butterfat secreted was determined. The cow received artificially dried alfalfa hay, corn silage and a grain mixture of the following composition: 4 parts, ground white corn, 2 parts ground oats, and 1 part linseed oil meal. The alfalfa hay, which was the principle source of the vitamin A (carotene) in the ration, was of excellent quality and was fed at uniform levels throughout the feeding trials. During the successive feeding periods, the grain mixture was modified by the addition of 10 per cent of the various oils to be tested. In addition to the three samples of soybean oil, cottonseed oil was used in the last feeding period to determine whether or not the vitamin A suppressing factor might also be present in this oil. The composition of the rations and the feeding schedule is given in table 1.

TABLE 1

*Showing the vitamin A activity and carotene content of the butters produced by the cow on rations containing the different oils*

Test period		Materials tested*	Butter	
			Vitamin A Units per gram	Carotene Micrograms per gram
No.	Days			
1	11	(Check)	33	7.3
2	11	Crude soybean oil	19	7.6
3	11	Soybean oil treated with special synthetic adsorbent	19	7.6
4	11	Soybean oil treated with Nuchar	31	7.7
5	11	Cottonseed oil	30	7.6

\* The basal ration consisted of artificially dried alfalfa hay as a source of vitamin A and a grain mixture consisting of 4 parts white corn, 2 parts oats and 1 part linseed oil meal.

Since earlier experiments (6) had demonstrated that the major effect of a ration upon the vitamin A content of milk takes place in the first ten or eleven days, eleven-day feeding periods were used in these tests. The milk during the last three milkings of each feeding period was collected, the cream separated, and churned into butter. The vitamin A potencies of these butters were determined by biological assay, using the technique previously described (1). Carotene determinations were also made upon these samples. Using the method of Schertz (7), with certain modifications (6), the samples were analyzed in a Bausch and Lomb spectrophotometer. The results of these experiments are given in table 1.

From the results of these experiments it can be seen that the butter made from the milk secreted by the cow fed the check ration (Period 1) possessed a high vitamin A value. This indicated that the potential vitamin A (caro-

tene) values of these rations were sufficiently high to insure the production of very potent butter. The lower vitamin A of the butter secured when crude soybean oil was added to the grain ration (Period 2), demonstrated the presence of the vitamin A suppressing factor in the crude soybean oil used in these experiments.

The low vitamin A potency of the butter secured in Period 3 indicates that the synthetic adsorbent did not adsorb this factor to any appreciable extent. When the cow was fed the oil treated with Nuchar (Period 4), the vitamin A potency of the milk returned to approximately the same value as that of the check ration, indicating that this adsorbent was effective in removing this factor. The comparative test with cottonseed oil indicates that this suppressing factor is not present in this oil in significant amounts.

Since a specially treated soybean oil did not suppress the vitamin A value of the butter when fed to the cow, this substantiates our earlier statement that the vitamin A suppressing action of soybean oil is not due to the oil itself but to some substance dispersed in it.

It is to be noted from the data given in table 1 that the carotene values for the different butter samples are rather uniform regardless of the vitamin A potencies of the butters. This seems to indicate that the suppressing factor interferes with the secretion of vitamin A per se in milk rather than the carotene. This peculiar effect has been observed repeatedly in earlier experiments. Furthermore, it has been observed that the carotene values as well as the vitamin A values may be lowered by additions of large amounts of either soybean oil or soybeans to the ration.

Although the Nuchar appeared to adsorb the active principle from the soybean oil, these experiments did not preclude the possibility that the factor might have been inactivated by the carbon rather than adsorbed. To test this possibility further tests were made upon the carbon. If the factor was adsorbed on the carbon, it should be highly concentrated upon the carbon and possibly might be removed by proper solvents. The removal of the active principle was attempted by extraction with acetone. The extract, after the removal of the acetone, contained much of the adsorbed oil, some sterol glucosides, coloring matter and other substances. The extracted carbon was dried and feeding tests made with both the extracted carbon and the extract as outlined in table 2.

Two Guernsey cows were given the check ration during the first period. In the second period, one cow received the extracted adsorbent and the other the extract in daily amounts equivalent to that used in the treatment of 0.8 lb. of oil. From the assays of the butter produced at the end of each feeding period, it is evident that the vitamin A suppressing factor was associated with the extracted carbon and that the extraction with acetone failed to remove any appreciable amount of this factor.

In order to eliminate the possibility of this suppressing effect being due

TABLE 2

*Giving the vitamin A activity and carotene content of butters produced by the cows on rations containing the adsorbent residue and extract from the residue*

Test period		Cow No.	Materials tested*	Butter	
				Vitamin A	Carotene
No.	Days			Units per gram	Micrograms per gram
1	21	535	(Check)	32	5.8
1	21	521	(Check)	33	5.7
2	21	535	Adsorbent residue	16	5.8
2	21	521	Extract from adsorbent	34	5.8

\* The basal ration consisted of artificially dried alfalfa hay as a source of vitamin A and a grain mixture consisting of 4 parts white corn, 2 parts oats and 1 part linseed oil meal.

to the carbon itself and not to an active principle adsorbed upon it, feeding tests were made with the addition of the original Nuchar to the ration of two Ayrshire cows. The results of this experiment are given in table 3. Since it was found that the addition of Nuchar to the ration of the cows was without effect upon the vitamin A value of the butter produced, it must be concluded that the treated carbon in the previous experiment held the active principle. Furthermore, it must be concluded that in the treatment of soybean oil with Nuchar the action of carbon is due to adsorption rather than to inactivation of the vitamin A suppressing factor.

TABLE 3

*Showing the vitamin A activity and carotene content of the butters produced by the cows on check rations and on a ration containing Nuchar*

Test period		Cow No.	Materials tested*	Butter	
				Vitamin A	Carotene
No.	Days			Units per gram	Micrograms per gram
1	21	159	(Check)	32	4.3
		166			
2	21	159	Nuchar	33	4.3
		166			
3	21	159	(Check)	33	4.2
		166			

\* The basal ration consisted of artificially dried alfalfa hay as a source of vitamin A and a grain mixture consisting of 4 parts white corn, 2 parts oats and 1 part linseed oil meal.

#### SUMMARY

- Crude soybean oil was treated with two different adsorbents in an attempt to remove the vitamin A suppressing factor which interferes with the transference of the vitamin A activity of feed to the butterfat secreted by dairy cows.

2. It was found that Nuchar removed the factor from the oil while the special sodium aluminum silicate adsorbent was without effect.
3. The action of the Nuchar was proven to be that of adsorption rather than inactivation.
4. Acetone did not elute the active principle adsorbed on Nuchar although acetone did extract oil, sterol glucosides, coloring matter and other substances.

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# VARIATION IN THE OXIDATION-REDUCTION POTENTIAL AS A CAUSE FOR THE OXIDIZED FLAVOR IN MILK

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## INTRODUCTION

Milk is consumed because of its nutritive value and its sweet and wholesome flavor. Any abnormal flavor affects its consumption and hence is an important economic problem.

The "oxidized" flavor which will be considered in this paper has been known by a number of terms. Those most commonly used have been "oxidized," "cappy," "cardboard," and "oily." The terms "cardboard" and "cappy" were used because early workers thought that the milk bottle cap was the source of the flavor, while the other terms were used to characterize a result which workers associated with the oxidation of fats.

The origin of the flavor concerned is not known, but its development seems to be associated with an oxidation phenomenon. Though the term "oxidized" has been used it has no definite meaning, but will be used here to denote the off flavor which develops in some milks, with or without metallic contamination, when they are held at 10° C., or below, in an atmosphere containing approximately 2½ per cent of oxygen.

The presence of this flavor has caused concern in Central Europe for a number of years. Recently it has become of increasing importance in the United States. Some authorities are of the opinion that the flavor has always been present, but that it has been masked by more pronounced flavors; others think that the advances in refrigeration and methods of handling milk are indirectly responsible for the increased prevalence of the flavor.

Hammer and Cordes (1) first discussed the development of a "tallowy" flavor in milk. They found that the flavor was caused by the action of light. In a later publication Frazier (2) came to the same conclusion. These results have been confirmed by Tracey *et al.* (3) and Doan and Myers (4).

Metals, especially copper, were considered to be important factors. Their effect has been studied by Guthrie and Roadhouse (5), Golding and Feilman (6), Hunziker and Hosman (7).

Kende (8) concluded that the flavor was caused by the oxidizing action of an enzyme which he called oleinase. The enzyme was so named because he thought the enzyme oxidized the oleic acid radical of the butterfat.

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Thurston *et al.* (9) concluded that the oxidation of lecithin was responsible for the flavor.

Sharp *et al.* (10) conclude that the flavor is caused by an enzyme similar to the enzyme that destroys ascorbic acid.

Period of lactation, feed, breed, and variation in ascorbic acid have also been given as possible explanations of the development of the flavor.

Greenbank (11) after study of the oxidation-reduction potential changes in milk came to the conclusion that the flavor is caused by a mild normal oxidation of a minor constituent.

#### EXPERIMENTAL

##### *Methods and Conditions Employed*

This work includes most of the factors which are known to be concerned in flavor formation and is extended to include other factors which might affect oxidation, with especial consideration of the oxidation-reduction potential of the medium. The results are given herewith.

(1) The milk used in these experiments came from the Bureau of Dairy Industry herd at Beltsville, Maryland. All samples were morning's milk delivered to the laboratories in pint bottles. The samples were treated within 6 hours after milking. Except for those cases wherein the effect of heating was the subject of investigation, the data are for raw milk.

(2) Pasteurization was by the holding method; 143° F. for 30 minutes in glass bottles.

(3) All samples, unless stated otherwise, were stored at 5° C. for 24 to 48 hours.

(4) Where copper was employed, the sulfate was used. The concentration is expressed as milligrams of copper per liter.

(5) The data presented in this paper are the result of a study of over 4000 samples from about 300 cows. The data presented are results representative of the samples studied. Data on many samples are eliminated for brevity and clarity.

(6) The Eh measurements were made by the potentiometric method.

(7) A plus (+) sign indicates an oxidized flavor; a plus and minus ( $\pm$ ) sign indicates that the judges could not determine if the flavor was present; a minus (-) sign indicates that the flavor was normal. The number of positive signs indicates the intensity of the flavor.

(8) In this work the classification of milk shall be similar to that of Thurston (12). That is, "spontaneous" milk, which does not require copper to develop the flavor; "susceptible" milk, which requires copper to develop the flavor; "non-susceptible" milk, which does not develop the flavor after the addition of small amounts of copper.

(9) An individual sample is milk from only one cow.

(10) The data on flavor are the results obtained by two judges, neither

knowing the results of the other, and neither having any knowledge of the treatment of the milk.

(11) As many data as possible were obtained on individual samples to eliminate as many variables as possible.

(12) Where possible the magnitude of the variables was changed gradually from zero to the maximum permissible.

(13) Every effort was made to handle the samples in the same manner each day. This was necessary because it has been shown that agitation and short periods at room temperature cause appreciable changes.

### *The Effect of Metals*

Contamination of dairy products by metals is known to produce abnormal flavors. Copper and iron have been found to be the greatest offenders in this respect. To determine the relative effect of these metals a series of samples of susceptible milk were contaminated by different concentrations of the metals. Iron was added in the form of the ferrous ( $Fe^{++}$ ) and ferric ( $Fe^{+++}$ ) ion. Copper was added as the cupric ( $Cu^{+}$ ) ion. After treatment the samples were stored at  $5^{\circ} C.$  and judged at the end of 24 and 48 hours. Table 1 shows the effect of the metals on the development of the flavor.

TABLE 1

*The effect of adding copper and iron on the development of the oxidized flavor in milk*

Metal added	Mg. per liter	Flavor of milk stored	
		24 hours	48 hours
None	0.00	-	-
Copper	0.07	-	+
Copper	0.15	-	+
Copper	0.22	+	++
Ferrous (iron)	0.40	+	+
Ferrous (iron)	0.80	++	++
Ferrous (iron)	1.20	++	++
Ferric (iron)	0.40	+	-
Ferric (iron)	0.80	±	-
Ferric (iron)	1.20	-	-

These results indicate that copper is more active as a catalyst than is ferrous iron. Ferric iron in the higher concentrations used proved to be an inhibitor to flavor formation. Since the mechanism through which the catalyst may be effective is not known, an attempt was made to find if there was a correlation between changes in oxidation-reduction potential ( $Eh$ ), with the addition of metallic salts, and the tendency to flavor formation.

A number of samples of milk were selected among which were both susceptible and non-susceptible samples. The  $Eh$  of each was then determined. The same concentration of copper was added to each sample, a second  $Eh$  determination was made and the samples placed in storage. After

48 hours' storage the samples were judged to detect any change in flavor. The results are shown graphically in figure 1.

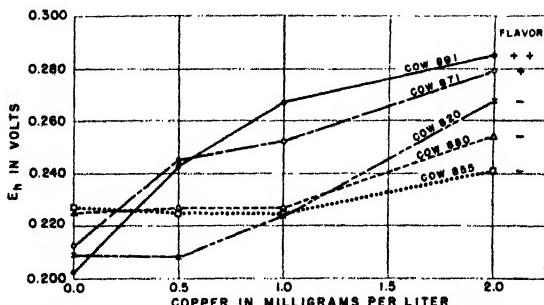


FIG. 1. The Eh's of milk samples to which different amounts of copper were added and their relation to the development of the oxidized flavor.

The values in figure 1 are in agreement with those of Hileman and Webb (13), who also noted a rise in the Eh of susceptible milk following the addition of copper. They found no correlation, however, between the oxidation-reduction potentials of milks from individual cows and their tendency to develop an oxidized flavor.

Our study of a large number of samples has shown that those exhibiting a relatively great increase in Eh after addition of copper are susceptible to flavor formation, while those with slight or no increase in Eh do not develop the flavor sufficiently to be detected by the sense of taste.

#### *Detection of Samples Susceptible to Flavor Development*

It would seem from the data just presented that it should be possible to detect susceptible samples through their change in Eh after the addition of copper. A large number of samples were studied and it was found that such a method was practical.

The test is made in the following manner. Twenty-five hundredth milligram of copper is added to 500 milliliters of milk. A copper sulfate solution containing one milligram of copper per milliliter is used. The Eh is then determined on the copper free and a copper containing sample. Both samples are then placed in storage for 6 hours and the Eh again determined. If the increase in Eh of the sample containing copper is 0.01 volt or more, the sample probably will become oxidized after 24 to 48 hours' storage at 5° C. Conversely, those samples which do not show 0.01 volt rise, probably will not become oxidized for a similar time of storage at 5° C. The values given in table 2 indicate the approximate magnitude of the Eh variation in samples susceptible and nonsusceptible to flavor development.

The test described in the previous paragraph can be used only on samples of milk which are relatively freshly drawn. The samples used were treated

within 6 hours of milking. Samples which have been shaken, or allowed to remain at room temperature for any appreciable time, may give varying results.

If a more severe control is desired, a larger amount of copper may be used. At no time should the amount be great enough to be detectable by taste or to promote other "oxidized" flavors.

These results seem to indicate that the susceptibility of a milk to flavor formation may be correlated with the ease with which its Eh changes in the

TABLE 2

*Detecting susceptible samples of milk by means of the increase in Eh after the addition of equal amounts of copper*

Cow No.	Eh in volts <sup>1</sup>			Flavor
	Plain	Copper added	Increase	
862	0.290	0.303	0.004	-
1272	0.272	0.289	0.017	+
1458	0.259	0.269	0.010	+
1355	0.247	0.253	0.006	-
1265	0.232	0.235	0.003	-

<sup>1</sup> 6 hours after addition of Cu.

presence of copper salts. The oxidation-reduction potential (Eh) is complicated by the poisoning action, which is, expressed in simple terms, the resistance of the milk to change in potential. This poisoning action corresponds to the buffering effect observed in measuring hydrogen ion concentration or pH. It would seem, therefore, that samples which are well poised show little change in Eh and hence do not promote oxidation reactions. Poorly poised samples, on the other hand, readily increase in Eh value, thereby promoting oxidation changes. Those samples which do not require copper to develop the flavor have much higher Eh values after the addition of copper than samples which require copper.

Many differences caused by variations in electrodes and apparatus may be compensated for by correlating the development of the flavor with the rise in Eh of susceptible samples.

#### *The Cause of Variations in Susceptibility of Individual Milks*

The variation in susceptibility of the milk from different cows has been one of the facts difficult to explain. Kende (8) explained it by the absence of the enzyme or by variations in the amount of reducing substances. The variation would seem to be more difficult to explain if in keeping with this theory the substance causing the flavor is always present. The variation is explained in this paper by the use of one variant—the poisoning action of the milk. If a sample is well poised at a low Eh the flavor will not develop. If poorly poised, the sample increases in Eh with contamination and the

minor constituent oxidizes to give the oxidized flavor. The effect of differences in handling and insignificant contaminations are reflections of poor poising.

Using poising as a criterion and Thurston's classification (12), spontaneous milk may be very poorly poised, susceptible milk poorly poised, and nonsusceptible milk well poised. That is, this classification may be expressed in terms of the poising action of the milk.

### *The Effect of Different Feeds*

Kende (8) found that feeding of green hay, or adding water extracts of green hay to the milk prevented development of the flavor. He assumed that the reducing substances in the green feed acted as antioxidants, thus preventing flavor formation.

Data are presented in table 3 on milk from cows that had been on dry feed for six months or more and were then put on pasture. Samples of the milks had been collected, stored, and scored prior to pasturing of the cows, and a similar procedure was followed at intervals during pasturing.

TABLE 3  
*The intensity of the oxidized flavor of milk from cows on pasture for varying periods of time*

Cow No.	Flavor <sup>1</sup>			
	0 wk. on pasture	5 wk. on pasture	10 wk. on pasture	16 wk. on pasture
N-429	++	+	++	++
N-219	++	-	-	-
N-419 <sup>2</sup>	+++	+++	+++	+++
A-103	+++	-	-	-
A-108	++	-	-	-

<sup>1</sup> 1 mg. of copper per liter.

<sup>2</sup> Recently fresh.

According to these data the development of the flavor is not always inhibited by feeding green feed. However, its beneficial effects are indicated by the fact that in every case there was a decrease in flavor intensity by the end of the fifth week. In experiments previously recorded, it has been shown that in susceptible milk samples there is an increase in the Eh after the addition of copper. Since this does not occur with certain samples of milk from cows that have been placed on pasture, a logical deduction would be that in those cases the poising action of the milk has increased. A study was, therefore, made to determine the variation in poising action when animals were placed on pasture, and the time that might be necessary to bring about the change. The data are given in figure 2.

Before the pasturing of cows 820, A-103, and A-99, the addition of 1 mg. of copper to a liter of their respective milks caused an increase in the Eh

values, and an oxidized flavor developed in each sample. After the cows had been on pasture for two weeks the addition of a similar amount of copper caused very little change of the Eh, and no flavor developed in the milks. The milk from cow A-105 changed little in Eh value after the addition of copper, even after the animal went on pasture. The results obtained seem to indicate that milks vary in their poising action. On this basis it may be said that the milk from cow A-105 was so well poised that addition of copper or reducing substances did not affect the Eh value greatly. The milks from cows 820, A-103, and A-99, on the other hand, may be said to have been poorly poised before the cows were put on pasture. Should the poising

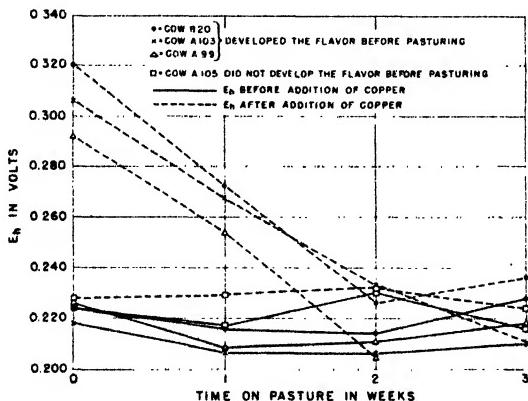


FIG. 2. The Eh of milk from cows at various stages of pasturing, before and after the addition of copper, and the effect upon the development of the oxidized flavor.

action of a milk be strong at a high potential, the flavor will develop while the cow producing the product is on pasture as well as when on dry feed except possibly for a slight difference in its intensity. The governing factor in the tendency of a milk to flavor development seems, therefore, to be its poising action.

#### *The Effect of Different Storage Temperatures on the Intensity of the Oxidized Flavor in Milk*

It is a well-known fact that the oxidized flavor developed by milks held in storage increases in intensity with decreases in storage temperatures. This is also evident from data obtained by Bell (15) upon frozen evaporated milks. These data are given in table 4.

Those samples stored at  $-17^{\circ}$  C. developed an oxidized flavor sooner and retained the flavor for a longer period than did those stored at  $-7^{\circ}$  C. The explanation for this fact lies probably in the effect of dissolved oxygen at different temperatures upon the relative rates of two or more successive

TABLE 4

*The effect of time and temperature of storage on the development of the flavor*

Storage (weeks)	Flavor	
	Storage temperature	
	-7° C.	-17° C.
0	good	good
1	good	oxidized
2	oxidized	oxidized
3	oxidized	oxidized
4	oxidized	oxidized
5	sl. oxidized	oxidized

reactions that may be involved in the formation and destruction of the flavor. (See discussion.)

#### *The Effect of Variation in the Time of Adding Metals on the Intensity of the Flavor*

It is a well-established fact that metallic contamination before pasteurization does not produce as intense an oxidized flavor as contamination after pasteurization. Brown *et al.* (14) were the first to point out this fact, but they gave no explanation for this phenomenon.

The conclusions of Brown *et al.* (14) have been confirmed through a number of experiments on samples to which copper had been added before and after pasteurization treatment. Other experiments were carried out to determine the relationship between flavor formation and the changes of potential when copper is added before and after pasteurization. The results are shown in figure 3.

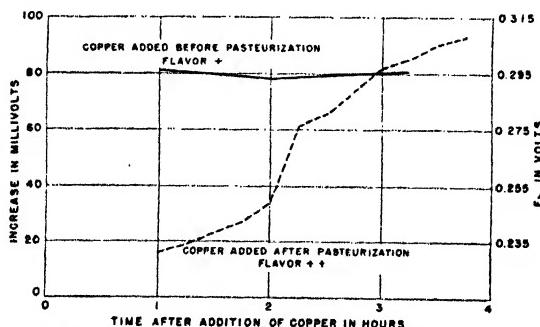


FIG. 3. The effect of the time of addition of copper on the development of the oxidized flavor and the Eh.

The Eh value of the milk sample to which copper was added before pasteurization changed but slightly after the sample was placed in storage, while that of the sample contaminated after pasteurization rose gradually

throughout the storage period and finally attained a value greater than that of the former sample. The fact that the sample to which copper was added before pasteurization does not reach as high an Eh value as the sample treated with copper after pasteurization is probably because of the formation of copper protein salts during heating.

### *The Effect of Heating*

The inhibition of flavor production caused by the heating of milk has in most cases been ascribed to the destruction of a specific enzyme "oleinase," described by Kende (8) and so named by him because he thought it promoted the oxidation of the oleic acid radical of the fat glycerides. Sharp (10) holds a similar view and concluded further that this enzyme is similar to the enzyme that oxidizes ascorbic acid, and that both enzymes are destroyed by heat. The heat treatment of milk necessary to prevent flavor development is 185° F. (85.0° C.) for 5 minutes, according to Kende (8); 170° F. (76.6° C.) for 10 minutes, according to Sharp (10); and heating to 170° F. (76.6° C.), according to Dahle (16).

Aside from the isolation by Kende (17) of an albumin-globulin fraction, which he claims contained the enzyme, no work has been done to prove its existence. Its presence has generally been assumed and the effect of heat has been ascribed to the inactivation of an enzyme and the production of reducing substances.

To determine the effect of heating milk upon its tendency to produce flavors, the following experiments were performed. After determination of the Eh of a raw milk, samples of it were heated to different temperatures, cooled, and the Eh of each determined. The results are given in table 5.

TABLE 5  
*The effect of heating milk at different temperatures upon the Eh*

Time of heating	Heating at 63° C.		Heating at 70° C.		Heating at 85° C.	
	Change in volts	Eh	Change in volts	Eh	Change in volts	Eh
min.						
0		+ 0.273		+ 0.273		+ 0.273
1	+ 0.005	+ 0.278	- 0.011	+ 0.262	- 0.033	+ 0.240
5	- 0.002	+ 0.271	- 0.025	+ 0.248	- 0.042	+ 0.231
10			- 0.048	+ 0.225	- 0.048	+ 0.225
15	- 0.008	+ 0.265				
30	- 0.017	+ 0.256				

Heating, at those temperatures shown by other workers to inhibit flavor formation (170° F. (76.6° C.) and 185° F. (85° C.)), markedly decreases the Eh of the milk, thereby indicating a stronger reducing tendency. Though the Eh was not decreased so greatly in the samples heated at 145.2° F. (63° C.) for 30 minutes as it was in those samples heated at 158° F.

(70° C.) and 185° F. (85° C.), there was no indication from these data or data on a great number of other samples studied that this heating increased the susceptibility to flavor formation. These data are in contradiction to those of Dahle (16) and others who have found that pasteurization promotes the development of flavor. From the above given data it seems probable that the prevention of vitamin C destruction and flavor development may be caused by the decrease in Eh, that is, a greater reducing tendency of the system.

#### *The Effect of Bacterial Growth*

The inhibiting action of bacterial growth was one of the first observations made by early workers in the field. Kende (17) and Dahle (16) attribute this action to the formation of reducing substances.

In order to study the effect of bacterial growth upon the development of flavors, samples of a susceptible milk were stored at different temperatures to obtain differing amounts of growth. Bacterial count and Eh determinations were made and samples cooled or warmed to the temperatures indicated in table 6. After 48 hours' storage at these temperatures the bacterial count and the Eh were again determined and the samples scored for flavor. The results are given in table 6.

TABLE 6

*Effect of bacterial growth on the Eh and the development of the oxidized flavor in milk*

Temperature ° F.	Eh	Bacterial count	Flavor
41 (5° C.)	0.341	5,800	+++
50 (10° C.)	0.321	59,000	++
57.4 (14° C.)	0.307	1,080,000	+
68.0 (20° C.)	0.165	109,000,000	-

As was to be expected, no flavor developed in the milk stored at 68° F. (20° C.), in which the Eh value dropped to 0.165 volts. The samples at 57.4° F. (14° C.), 50° F. (10° C.) and 41° F. (5° C.) increased in intensity, respectively. With this increased intensity there was a respective increase in Eh.

#### *The Effect of Variation in Ascorbic Acid*

The variation of the ascorbic acid content of milk has been suggested as a cause for the oxidized flavor by Kende (8); Dahle (16), and Sharp (10), have also studied its effect in this respect.

Ascorbic acid (Vitamin C) is a reducing agent and the Eh in water solution as determined by Ball (18) appears to be low enough to inhibit development of the flavor, were it caused by an oxidation.

Experiments were, therefore, conducted to determine if the amounts of ascorbic acid necessary to inhibit flavor formation would fall within the range of values for the amounts of this compound normally present in milk.

Most of these experiments were conducted with raw herd milk, though some susceptible and non-susceptible samples from individual cows were used. The ascorbic acid was added in crystalline form and the samples judged at the end of 48 hours in storage.

TABLE 7

*The effect of ascorbic acid (vitamin C) on the development of the oxidized flavor in milk*

Cow No.	Flavor when ascorbic acid was added at the rate of			
	0 mg. per liter	10 mg. per liter	20 mg. per liter	40 mg. per liter
N-419	+	+	±	-
N-429	+	+	±	-
A-65	-	-	-	-
N-219	-	-	-	-

The results indicate that the presence of ascorbic acid can prevent the production of oxidized flavor; however, the amounts required are greater than the probable normal variations in this constituent, 22.2 to 29.2 mg. per liter, Whitnah (19).

#### *Removal of the Oxidized Flavor in Milk by a Reducing Agent*

The inhibition produced by bacterial growth, green feed, and heating is caused by an increase in reducing substances, as reflected in the lower Eh values of the milk.

An attempt was made to ascertain if the oxidized substance responsible for the flavor could be rendered innocuous by the action of a reducing agent. To do this, different amounts of sodium sulfite ( $\text{Na}_2\text{SO}_3$ ) were added to samples which had just become positive and the samples stored at 4° C. for 24 hours. After storage the Eh of the samples was determined and the samples judged for flavor. The results are shown in table 8.

TABLE 8

*The effect of a reducing agent ( $\text{Na}_2\text{SO}_3$ ) on the oxidized flavor in milk*

Mg. $\text{Na}_2\text{SO}_3$ per liter	Eh	Flavor	
		Judge No. 1	Judge No. 2
0	0.304	++	++
1	0.281	++	+
2	0.273	±	+
4	0.241	±	+
8	0.220	-	-
16	0.191	-	-

As the concentration of sodium sulfite increased the Eh decreased and the flavor became less pronounced and at a concentration of 8 milligrams per liter the flavor was not discernible by the sense of taste. As previously

indicated, this type of experiment cannot be carried out on milk in which the flavor has been taken up by the fat and protein. This is not absolute proof that the precursor is regenerated by reduction but seems to indicate that such is the case.

*The Effect of the Removal of Air on the Development of the Oxidized Flavor in Milk*

According to Sharp (10) removal of air inhibits the development of the oxidized flavor in milk. Studies made in these laboratories indicate that the development of the flavor is inhibited if the concentration of copper is low. This inhibition is probably caused by the removal of oxygen which reduces the Eh value of the milk. The addition of a high concentration of copper increases the Eh and the flavor develops.

*The Effect of Light*

In the experiments recorded here an attempt was made to find the effect of light upon the development of the flavor and upon the Eh of the milk. Samples of non-susceptible milk with and without copper were exposed for varying lengths of time to the diffuse light from a western exposure. At the end of the exposure the samples were placed in storage at 41° F. (5° C.) for 48 hours and were scored. The Eh values were determined before and after exposure. The results are given in table 9.

TABLE 9

*The effect of diffuse light on the Eh and the development of the oxidized flavor in milk*

Exposure	Copper per liter	Eh	Flavor
hr.	mg.	volt	
0	0.5	0.234	-
3	0.0	0.228	-
3	0.5	0.257	++
6	0.0	0.242	-
6	0.5	0.246	+

The results in table 9 agree with those of experiments with several thousand samples, in that ordinary exposures to light of uncontaminated samples do not affect the flavor. In the presence of copper an exposure of 3 hours promotes the development of the flavor and increases the Eh more than does 6 hours' exposure. In all probability light in the presence of copper is such a strong oxidizing agent that prolonged exposure causes a reaction similar to that of the ferric ion shown in table 1.

After the study of the effect of diffuse light an attempt was made to determine the action of direct sunlight. In this experiment a number of samples of non-susceptible milks were exposed to the direct February sun in pyrex flasks. Pyrex was used because of its high transmission in the ultra-

violet end of the spectrum. Some of the samples contained copper. The approximate temperature was 32° F. or 0° C. During exposure and at definite intervals afterward, the Eh of the samples was determined. After the exposure the samples were held at 5° C. and judged at the end of 48 hours' storage. Figure 4 shows the effect of sunlight on the flavor and Eh of non-susceptible milk.

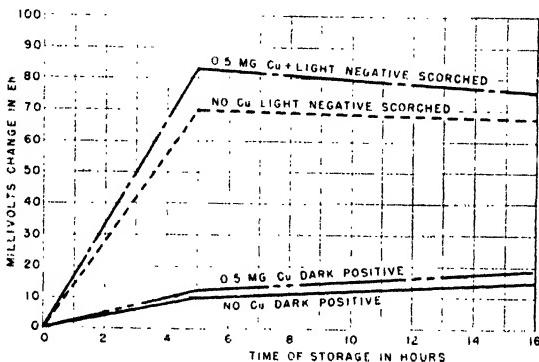


FIG. 4. The effect of sunlight on the Eh and the development of the oxidized flavor in milk.

Direct sunlight with its shorter wave lengths is so strong an oxidizing agent that it increases the Eh so greatly and so rapidly that a scorched flavor results. Experiments described elsewhere on the effect of hydrogen peroxide and contamination before pasteurization indicate that a rapid rise in Eh is not conducive to the development of the flavor.

#### *The Promotion and Inhibition of the Oxidized Flavor in Milk by Oxidizing Agents*

It has been shown by Greenbank (11) and also in table 1 that the oxidized flavor in milk may be destroyed by increasing the tendency toward oxidation. In the experiments quoted no attempt was made to measure the oxidation-reduction potential of the milk in question.

An attempt was then made to form the oxidized flavor by a low concentration of the oxidizing agent and destroy the flavor by a higher concentration of the chemical. The changes in intensity of oxidation were followed by means of the increase in the Eh. A susceptible milk was treated with different concentrations of hydrogen peroxide (30 per cent) and placed in storage for 4 hours. The samples were then removed, the Eh determined, and the samples judged. The results of this experiment are given in table 10.

The results were repeated a number of times on different samples. No two samples gave exactly the same results but all showed the same trend.

TABLE 10  
*The promotion and inhibition of the oxidized flavor formation in milk  
by hydrogen peroxide*

Concentration of of 30% hydrogen peroxide per liter	Eh	Flavor	
		Judge No. 1	Judge No. 2
cc.	volt		
0.00	0.231	-	-
0.03	0.291	+	+
0.06	0.354	+	+
0.12	0.392	++	++
0.20	0.431	+	+
0.50	0.410	-	-

*The Effect of Variations in the Hydrogen-ion Concentration*

The Eh of reversible oxidation-reduction systems change with variation in their respective H-ion concentrations. The effect of variations in the H-ion concentration of milks susceptible to flavor development was promoted or retarded in accordance with known variations in H-ion concentration. Samples of "spontaneous" milks were treated with lactic acid or sodium hydroxide to obtain a desired pH, stored at 41° F. (5° C.) and scored at 24- and 48-hour periods. The data are given in table 11.

TABLE 11  
*The effect of varying the pH in the same milk on the development of the oxidized flavor*

pH	Flavor	
	After 24 hr. storage	As ranked by judges after 48 hr. storage
6.90	-	1
6.79	-	2
6.65	-	3
6.55 (control)	+	4
6.49	+	6
6.32	+	5
6.25	+	6
6.01	+	5

The data indicate that a decrease in the H-ion concentration of the milk equivalent to 0.10 pH unit is sufficient to inhibit the development of flavor for 24 hours. Though the flavor develops in 48 hours, those samples with decreased H-ion concentration produced less flavor than any of the samples of increased acidity.

These conclusions are in agreement with former results of Greenbank (11) confirmed by Anderson (21). The latter author, however, explained the effect on the supposition that the neutralizer is an activator of an enzyme which destroys flavor, rather than upon the theory of catalysis of oxidation by the OH-ions.

*The Effect of Antioxidants*

The use of antioxidants is so general today that any study of a chemical oxidation would not be complete without their application. The mechanism of antioxygenic action is not well understood; therefore, many of the properties used to determine whether or not a substance will oxidize cannot be used. However, an attempt was made to determine if the antioxidant hydroquinone would counteract the action of copper. A number of samples of a susceptible milk were treated with hydroquinone or copper, and certain samples were treated with both. After treatment the samples were placed in storage and judged at the end of 48 hours. Table 12 shows the effect of hydroquinone on the development of the flavor.

TABLE 12

*The effect of hydroquinone on the development of the oxidized flavor in milk*

Treatment		Flavor	Score
Hydroquinone mg. per liter	Copper mg. per liter		
0.0	0.0	++	4
0.5	0.0	+	3
1.0	0.0	-	1
0.0	0.5	++	6
0.5	0.5	++	5
1.0	0.5	-	2

Hydroquinone proved to be an effective antioxidant in preventing the development of the flavor. Two parts of hydroquinone were required to counteract the action of one part of copper.

Incidentally, it may be stated that although some samples did not develop the flavor, those containing only hydroquinone were always judged of superior quality. This observation would seem to indicate that there may be other types of oxidation which affect the flavor and which are not considered highly objectionable.

*Controlling the Development of the Flavor by Variations in the Oxidation Reduction Potential (Eh)*

It has been shown in experiments heretofore described that heating reduces the Eh and inhibits the development of the oxidized flavor in milk. Since the poising action of milk varies, it is possible that the respective change in Eh value resulting from heat treatment may vary in degree. In some cases the poising action may be so great that the decrease in Eh upon heating may be too small to prevent flavor development even when copper is present. To determine if this is the case, a number of samples were selected and heated to 185° F. (85° C.) for 5 minutes. After cooling, copper was added in different concentrations and the samples stored at 5° C. for 48 hours. The results are given in table 13.

TABLE 13

*The effect of heating milk to 185° F. (85° C.) for 5 minutes on copper as a catalyst for the development of the oxidized flavor in milk*

Cow No.	Flavor when copper was added in the concentration of				
	0 mg. per liter	0.15 mg. per liter	0.2 mg. per liter	0.4 mg. per liter	0.8 mg. per liter
A-110	-	-	-	-	+
N-222	-	-	-	-	+
N-415	-	-	-	-	-
N-418	-	+	+	+	+
N-419	-	+	+	+	+
N-420	-	+	+	+	+

The data seem to indicate that any sample may become oxidized if sufficient copper is added. They also seem to indicate that the reducing substances formed by heating may be oxidized by the copper and the Eh increases to a point which favors flavor development. An attempt was made to test this hypothesis.

Heating raw susceptible milk to 158° F. (70° C.) for 10 minutes has been shown to decrease Eh and inhibit development of the flavor. The addition of copper to raw "susceptible" milk has been shown to increase the Eh and promote the development of the flavor. Since heating reduces and the addition of copper increases the Eh, it should be possible to heat a copper-containing milk and obtain the same Eh as the raw milk. If an increase in Eh alone is responsible for the development of the flavor, the addition of more copper to the heated copper-containing milk should cause an increase in Eh and promote development of the flavor.

The results in table 14 show that if sufficient copper is added to a heated sample, it will develop the flavor.

TABLE 14

*Controlling the development of the oxidized flavor in milk by varying the Eh*

Sample No.	Treatment	Eh volt	Change in Eh volt	Flavor
1	Raw	0.222*	.000	-
2	Raw + 1 mg. Cu per l.	0.232*	+.010	+
3	Heated milk + 1 mg. Cu per l.	0.192*	-.030	-
4	1 mg. Cu per l. added before heating	0.222	-.000	-
5	Same as (4) + 1 mg. Cu after heating	0.235*	+.013	+

\* After 6 hours' storage.

By raising or lowering the Eh value of the same milk, it has been possible to promote or inhibit the development of the oxidized flavor.

The relation of the changes in the Eh of the milk to the development of the oxidized flavor and the probable reaction are shown in figure 5.

## DISCUSSION

The results of the experiments described in this manuscript seem to indicate that the oxidized flavor of milk is caused by a mild oxidation of some minor constituent or constituents. This conclusion is supported by the fact that in a reducing environment, such as that caused by the mechanical removal of air, removal of oxygen by bacterial growth, or presence of antioxidants, etc., the flavor does not form readily. On the other hand, conditions

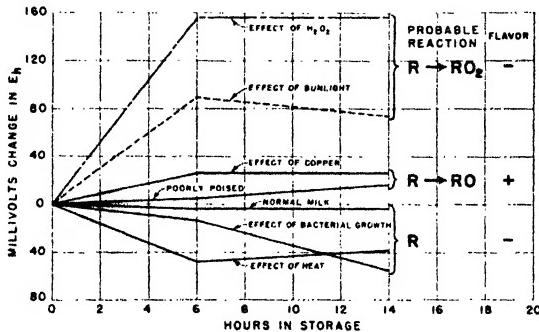
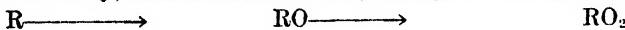


FIG. 5. Summary diagram, based on a number of results obtained showing the relationship of variation in the oxidation reduction potentials of milks to the development of the oxidized flavor.

favoring mild oxidation, such as low temperatures of storage, presence of certain metals that catalyze oxidation reactions, or subjection to the action of diffused light in the presence of metals, action of small amounts of hydrogen peroxide, etc., favor flavor formation. However, flavor development is also inhibited by heating the milk, by the action of certain concentrations of hydrogen peroxide or by the presence of oxygen (aeration) etc. Conditions favoring oxidation seem therefore to be able to promote or inhibit flavor formation, depending upon their intensity. This fact seems to be most satisfactorily explained through the assumption that two stages of oxidation are involved; namely, oxidation of the compound or compounds involved to intermediate compounds which possess the flavor or to more completely oxidized compounds that are tasteless.

Schematically, such an oxidation may be represented as follows:



No oxidized flavor      Oxidized flavor      No oxidized flavor

Thus, when mild oxidizing conditions exist the oxidized flavor develops. Under more strongly oxidizing conditions the reaction proceeds to a more complete stage and the oxidized flavor does not develop.

Furthermore, the results obtained with milks stored at different temperatures may best be explained upon the assumption that the rates of reaction of the stages of the oxidation are different and are affected to different degrees by temperature changes.

Such a theory serves to correlate most satisfactorily the observations recorded here, as well as those made by other workers, concerning the development of the flavor without resorting to explanations based on assumed enzyme activity.

All of the major constituents of milk are relatively stable to mild oxidizing conditions, and especially under those conditions which are known to affect flavor formation. This would seem to eliminate the glycerides of the fat, casein, lactose and albumin, as precursors of the flavor.

Since the oxidation concerned seems to be affected greatly by mild oxidizing or reducing conditions, it seems that this state is most satisfactorily explained by the variations in oxidizing and reducing tendencies of the milk in question. That this may be the case is supported by the data presented. Under conditions which increase the oxidation-reduction potential of the milk, flavor development is favored. However, strong oxidizing action may inhibit the flavor development primarily through completion of the oxidation as heretofore explained. Conversely, a lowering of the oxidation-reduction potential favors inhibition of flavor formation. These facts indicate that there is a direct relationship between the potential of the system and the tendency of a milk to develop off-flavors. Copper is an ideal catalyst for flavor development because the Eh value of the milk when this metal is added is great enough to promote oxidation but not great enough to promote a rapid oxidation of the reaction concerned to completion. There seems also to be a variation in the poisoning action of different milks. Milks which do not readily develop the flavor seem to be well poised while those milks which develop flavor increase in potential with the addition of small amounts of copper and develop oxidized flavors. Heating the milk or feeding the animals producing the milk on green feeds seems to increase poisoning action, thereby inhibiting flavor development.

The fat has often been considered as a probable precursor of the compounds that are responsible for the flavor. Though changes in the iodine number of fats with changes in flavor have been observed by Dahle (16), and by Kende (8), no changes were observed by Brown *et al.* (22). The latter observation seems to be in accord with the work of Holm and Greenbank (23), who observed that fresh milk fat is relatively stable to spontaneous oxidizing action even at relatively high temperatures and that sufficient oxidation to produce tallowy flavor affects the iodine value but slightly. These facts seem to exclude the glycerides of fat as possible precursors of the compounds that are responsible for the flavor but does not necessarily exclude minor fat constituents, such as lecithin, which compound Thurston (9) has suggested is indirectly responsible for the flavor.

Oxidizing conditions which have little effect on the major constituents of milk are capable of inhibiting the development of the flavor in milk and the fact that the flavor develops to a greater intensity as the storage tem-

perature is decreased does not support the idea that a major constituent of the milk is concerned.

The theory that enzymic action is responsible for the flavor is not easy to support in view of the evidence presented; namely, that the flavor developed to a greater intensity at a storage temperature of 1.4° F. (-17° C.) than at higher storage temperatures, that flavor developed in milk samples heated to 185° F. (85° C.) for 5 minutes, and that flavor did not develop in aerated samples of the same milk (10).

On the basis of the proposed theory, the anomalous fact that flavor develops more rapidly and to a greater intensity in milk stored at 1.4° F. (-17° C.) than in milk stored at 19.4° F. (-7° C.) may be viewed as a result of a decreased rate of oxidation by dissolved oxygen to the more completely oxidized stage brought about by the lower storage temperature. The net result is an accumulation of the intermediate or flavored compound.

#### ACKNOWLEDGMENT

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## EQUILIBRIUM SOLUTIONS OF CERTAIN LACTOSE-SALT MIXTURES

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Although the solubility characteristics of lactose in water have been rather extensively studied (10, 14, 5), recorded data concerning the melassigenic properties of various salts are extremely meager (11). This lack of comprehensive or critical data has imposed certain limitations in adequately dealing with some industrial processes and developmental research in which the crystallization of lactose is directly or indirectly concerned. The literature discloses substantially only one series of data concerning the solubility of lactose in known salt solutions, namely, the work of Herrington (11) involving calcium chloride, which incidentally led to the isolation of a lactose-calcium chloride compound. Since the formation of calcium chloride compounds with other sugars has been reported (3, 1, 8, 16), the evidence might be interpreted to indicate a particular molecular configuration especially conducive to compound formation with this salt and possibly other salts with closely related properties. The purpose of the present work had a two-fold objective: first, to determine the influence of various salts upon the solubility of lactose, thereby ascertaining whether certain ions exhibited an orderly relationship in melassigenic properties; and secondly, to determine whether other calcium-halide compounds analogous to calcium chloride-lactose compounds could be formed under appropriately controlled conditions.

### EXPERIMENTAL

The lactose employed in this study was the alpha hydrate which fully conformed to the U. S. Pharmacopoeia specifications, of a degree of fineness which permitted passage through a 120-mesh screen. The salts were of the "chemically pure" and "tested purity" class with the exception of the calcium bromide and calcium iodide which were labeled "pure." No further purification was attempted. The following procedures were used in carrying out the solubility determinations:

(A) The salt solutions were prepared in graduated concentrations at five per cent intervals by adding the required amounts to 50 grams of distilled water. Salts containing water of crystallization were either dried to the anhydrous form or, when this procedure was impracticable, appropriate calculations were made to determine the equivalent of the anhydrous material. The standardized salt solutions were placed in screw-cap bottles, which in turn were attached to a vertical wheel slowly rotating within a

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constant temperature air bath maintained at 35° C. Following a period of agitation and tempering of the salt solutions, the refractive index was determined at the temperature of the air bath; all refractive index determinations required throughout the course of this study were made at this temperature.

(B) An excess of lactose was added to each solution and agitation at 35° C. resumed. At intervals of 24 hours the bottles were removed from the agitator and held in a stationary position in the air bath until the suspended undissolved lactose had settled. The refractive index was then determined; this determination served to disclose the amount of lactose which had dissolved during the 24-hour period. The method used to calculate the amount of lactose dissolved in the salt solutions is explained in section (C). A comparison of the values obtained on consecutive days indicated clearly when equilibrium had been reached, at which time further agitation was discontinued and a final refractive index determination made on the equilibrated solution free from suspended lactose. The concentration of lactose in at least one of the graduated series of each salt solution was then checked by other methods. The iodometric method of Kline and Acree (12), further studied and modified by Miller (13), was found useful where copper reduction methods were inapplicable.

(C) Standardized salt solutions containing known amounts of lactose were prepared and their refractive indices determined in the same manner as for the solutions of unknown lactose concentration. By employing these values as a basis of reference, the lactose concentration in the equilibrated solutions may be determined by interpolation or graphic means; the graphic method was used in these studies. Obviously the results will be in error if any salt has been removed from solution. In view of this possibility, the results were checked at strategic points by determining the lactose and/or salt concentration by other methods. For illustration, in the case of the calcium-halides which potentially form calcium halide-lactose complexes, calcium determinations were made in addition to the refractive index readings.

In preparing the standard salt solutions containing known amounts of lactose, it is necessary to prepare at least one solution at each salt concentration in which the lactose concentration is equal to or greater than the equilibrium value. In most cases this was readily accomplished by raising the temperature above 35° C. to effect solution, cooling at 35° C. and determining the refractive index before crystallization. Usually no difficulty was encountered because the solutions remained supersaturated for a considerable time. The refractive index determinations were made with an Abbé type instrument.

The results obtained with twenty-four salts are shown in table 1. The influence of certain sodium, calcium, and magnesium salts on the solubility

of lactose is shown graphically in charts 1 and 2, respectively. It is clearly evident that the salts investigated differ widely in their abilities to influence the solubility of lactose in water. The ions arranged in order of their effectiveness (based upon a 25 per cent salt concentration) in increasing the solu-

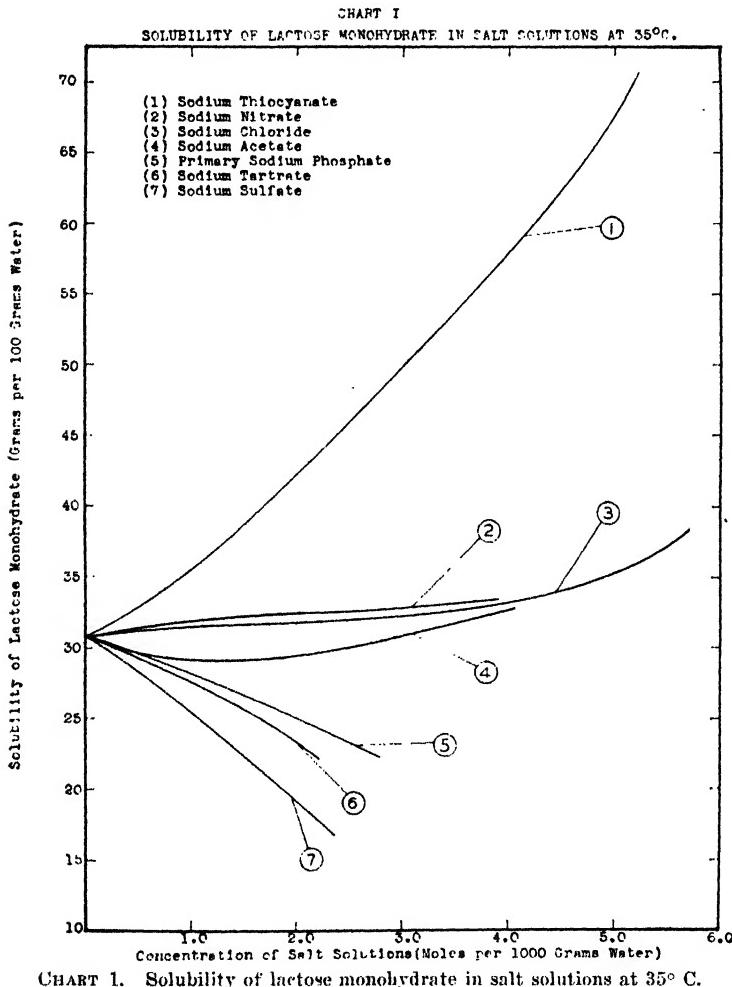


CHART 1. Solubility of lactose monohydrate in salt solutions at 35° C.

bility of lactose show the following sequence for the anions: thiocyanate, iodide, nitrite, bromide, chloride, nitrate, acetate, tartrate, dihydrogen phosphate, and sulphate. It is probable that the comparisons are of greater value when made on equimolar concentrations; such comparative values are graphically shown in charts 1 and 2. The order of effectiveness

of the cations appears to fall in approximately the following sequence: potassium, calcium, sodium, and magnesium.

It is of considerable interest to note that the sequence of the anion series conforms essentially to the series of ions compiled by Cooper (2) based

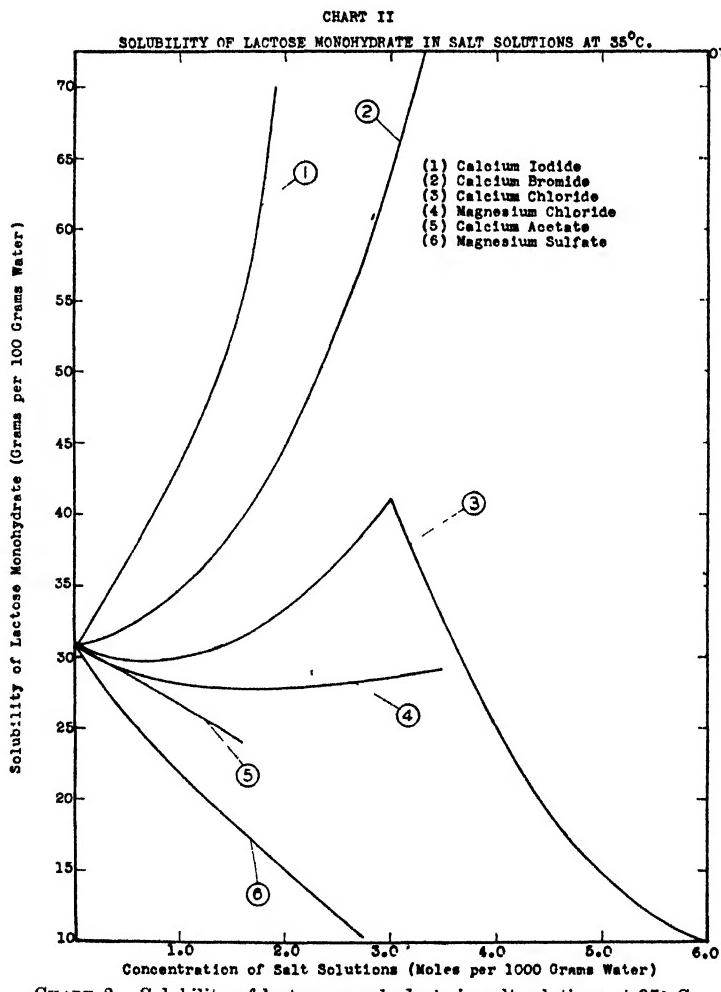


CHART 2. Solubility of lactose monohydrate in salt solutions at 35° C.

upon a calculation of the free energy of formation of the ions from their elements. His calculations place the common anions in the following order: cyanide, hydrosulphide, bromate, thiocyanate, chlorate, nitrite, perchlorate, iodide, cyanate, bromide, nitrate, chloride, iodate, fluoride, acetate, sulphite, bisulphite, fumarate, oxalate, sulphate, citrate and tartrate. Cooper points

TABLE I

*Solubility of lactose monohydrate in various salt solutions at 35° C.*

*A*—Concentration of salt solution in per cent.

R—Concentration of salt solution expressed as moles per 1000 grams water.

C—Solubility of lactose (grams lactose monohydrate per 100 grams water).

out that this series conforms in general to the lyotropic or Hoffmeister series. An orderly sequence of effectiveness of the different ions on colloidal phenomena such as the peptization of proteins (6), the flocculation of either hydrophobic or hydrophilic colloids (15, 7), and swelling pressures (4) has been demonstrated; certain ions causing a predominantly "solubilizing" effect and others a "precipitating" effect.

Hoffmeister (9) has explained the action of the ions on the basis of their abilities to monopolize the water in a solution. This offers a plausible explanation for the variation in the precipitating or salting-out effect of the ions but it is wholly inadequate to account for solubilizing effects. It is known that ions in the end of the series have a dehydrating effect because they adsorb water with great avidity but it cannot explain why the ions at the beginning of the series, which are weakly hydrated, drive lactose into solution. It seems feasible to postulate that the ions at the beginning of the series combine with lactose, the resulting compounds or complexes being more soluble than the lactose itself. This hypothesis is supported by the results obtained with calcium chloride and by those obtained with calcium bromide and calcium iodide as will be shown hereinafter.

The effect of calcium chloride on the solubility of lactose is of particular significance (chart 2). It is evident that at concentrations greater than approximately 25 per cent (3 moles per 1000 grams water) the apparent solubility of lactose drops off sharply, due presumably to the formation of a compound or complex. Herrington (11) has isolated such a compound and assigned the formula: alpha lactose ·  $\text{CaCl}_2 \cdot 7\text{H}_2\text{O}$ . This compound can be made easily as shown by the following example.

Starting with a solution of lactose ·  $1\text{H}_2\text{O}$ , 5 lbs.;  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ , 7 lbs. 13 oz.; and water, 9 lbs. 6 oz., 5 lbs. of the pure compound were readily precipitated. By adding amounts of lactose and calcium chloride to the mother liquor equivalent to the amount of compound crystallized out, second and third yields of the crystallized compound were obtained in 4 lb. 4 oz. and 4 lb. 14 oz. quantities, respectively. It appears that the process can be continued indefinitely with practically one hundred per cent recovery of the lactose and calcium chloride in the form of the complex. The crystals can be separated from the mother liquor by filtering and washing with a solution of 80 per cent acetone and 20 per cent water. The resulting product showed 62.1 per cent lactose and 6.90 per cent calcium by analysis, which conforms to the respective calculated values of 62.2 and 6.92 for a compound, lactose ·  $\text{CaCl}_2 \cdot 7\text{H}_2\text{O}$ .

An analogous compound, lactose ·  $\text{CaBr}_2 \cdot 7\text{H}_2\text{O}$ , has been prepared in a similar manner. Preliminary determinations indicated that the mole ratio of calcium bromide to lactose should be at least two to one to yield crystals of the complex. Starting with a solution of lactose ·  $1\text{H}_2\text{O}$ , 5 lbs.;  $\text{CaBr}_2 \cdot 6\text{H}_2\text{O}$ , 10 lbs. 8 oz.; and water, 6 lbs., 6 lbs. 3 oz. of a crystalline compound

were obtained. The product showed 53.9 per cent lactose and 6.36 per cent calcium by analysis which conforms to the calculated value of 53.9 per cent and 6 per cent, respectively, for the compound, lactose · CaBr<sub>2</sub> · 7H<sub>2</sub>O. By adding equivalent amounts of lactose and calcium bromide to the mother liquor 4 lbs. 13 oz. and 5 lbs. yields were obtained from the second and third crystallization, respectively. The crystals produced were exceedingly fine, slender prisms with "hip-roofed" ends apparently consisting of three faces. They were smaller and more soluble than those of the corresponding calcium chloride complex. When washed free from excess calcium bromide, drying was readily accomplished in ordinary atmosphere to about 6 per cent of free moisture; this may be removed by further drying at 70° C. The washing of the crystals is best accomplished with a solution of 80 per cent acetone and 20 per cent methyl alcohol.

In those studies concerning the solubility of lactose in calcium iodide solutions, it was revealed that the solubility increased rapidly with increasing concentrations of calcium iodide (chart 2). In a 40 per cent calcium iodide solution, about 82 parts of lactose are soluble in 100 parts of water. Greater concentrations of calcium iodide were employed in order to determine whether or not at some higher concentration a complex analogous to the lactose-calcium chloride and lactose-calcium bromide compounds might be formed. Aqueous solutions of calcium iodide were prepared in concentrations varying from 45 to 70 per cent. Lactose was added to these solutions in small increments until saturation at room temperature was reached. A small additional quantity of lactose was then added and the mixture warmed to effect complete solution. Upon cooling to room temperature crystalline precipitates appeared. Table 2 contains the tabulated observations correlating the solubility of lactose and character of the crystalline material obtained from supersaturated solutions with the concentration of the calcium iodide solutions.

TABLE 2

*Approximate solubility of lactose monohydrate in calcium iodide solutions and character of crystals formed in supersaturated solutions*

Calcium iodide solutions per cent	Solubility of lactose monohydrate at room temperature gm. per 100 gm. water	Crystals formed in supersaturated solutions
45	100	Alpha lactose
50	104	" "
55	120	" "
60	129	" " and compound
65	33	Lactose-calcium iodide (prisms)
70	Less than 20	" " "

It is evident that the deposition of the compound takes place at appropriate concentrations of calcium iodide and lactose. The necessary condi-

tions for crystallization are a concentration of 65 per cent or more of calcium iodide, and lactose slightly in excess of its solubility at the existing salt concentration. Successive batches of the compound were crystallized from the same mother liquor by maintaining the calcium iodide concentration at 65 per cent or above and making further additions of lactose if crystals of the compound failed to appear on standing; likewise, the method used for the production of the other lactose-calcium halide compounds was employed, namely, that of adding equimolar quantities of halide and lactose in the amount equal to the weight of the compound removed by crystallization. Several lots of the compound have been prepared from solutions containing 200 gm. alpha lactose, 350 gm. calcium iodide, and 200 gm. water. The product showed 52.2 per cent lactose, 5.81 per cent calcium, and 36.2 per cent iodine by analysis, which conforms to the calculated values of 52.2 per cent, 5.81 per cent, and 36.8 per cent, respectively, to the constituent components of a compound, lactose ·  $\text{CaI}_2 \cdot 3\text{H}_2\text{O}$ .

The removal of excess calcium iodide from the crystals is of primary importance in the production of a satisfactory and stable material. Iso-propanol was found to be eminently suitable for this purpose as the crystals are only sparingly soluble in it. In practice it has been found that three washings with iso-propanol followed by the same number of washings with 98 per cent ethyl alcohol produced clean crystals which could be readily dried and freed of alcohol at 70° C. in a vacuum oven.

Table 3 shows some of the physical characteristics and properties of each of the lactose-calcium halide compounds prepared according to the methods described.

TABLE 3  
*Certain properties of lactose-calcium halide compounds*

	Lactose · $\text{CaCl}_2 \cdot 7\text{H}_2\text{O}$	Lactose · $\text{CaBr}_2 \cdot 7\text{H}_2\text{O}$	Lactose · $\text{CaI}_2 \cdot 3\text{H}_2\text{O}$
Specific rotation	+ 32.6	+ 28.3	+ 27.4
Melting point	86-87°	98° (softens)	180° (decomp.)
Solubility in water at 35°*	52 parts	69 parts	86 parts
Solubility in ethyl alcohol	Insoluble	Insoluble	Very slightly soluble
Solubility in ethyl ether	Insoluble	Insoluble	Insoluble

\* Expressed as grams per 100 grams water.

The successful crystallization and isolation of lactose-calcium halide compounds provides evidence in support of the hypothesis that the observed marked increase in solubility of lactose in the presence of these salts is due in reality to the formation of a complex more soluble than the lactose itself. Whether similar complexes are formed with lactose and other salts which cause an apparent increase in effectiveness for maintaining lactose in solution, cannot be stated with finality on the basis of the data presented. The

evidence indicates that the separation, by differential crystallization, of such complexes as may be formed would involve definite knowledge and control of critical saturation levels of the lactose, salt and compound components of the multiple phase system.

#### SUMMARY

1. The solubility of lactose in solutions of twenty-four different salts has been determined. The comparative influence of the various salts places the ions in an order of arrangement conforming essentially to the series compiled by Cooper based upon free energy values, and to the Hoffmeister series.
2. The solubilizing effects noted are explained by postulating the formation of complexes or compounds which are more soluble than the lactose itself.
3. The lactose ·  $\text{CaCl}_2 \cdot 7\text{H}_2\text{O}$  compound previously reported has been prepared by simple means, and methods for preparing two other compounds, lactose ·  $\text{CaBr}_2 \cdot 7\text{H}_2\text{O}$  and lactose ·  $\text{CaI}_2 \cdot 3\text{H}_2\text{O}$ , are described.

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## SOME OBSERVATIONS ON THE ERADICATION AND CONTROL OF BANG'S DISEASE

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Research on Bang's disease has been in progress at the Storrs Agricultural Experiment Station since 1913.<sup>1</sup> The ground work for our present program of control was laid by Rettger, White, McAlpine, Johnson, and Chapman. Their early researches yielded several important contributions to the fundamental knowledge of the diagnosis and control of Bang's disease, involving periodic blood testing and segregation and removal of infected animals. Of special value to such a program were their observations; (1) that calves, with rare exceptions, remain resistant to an established infection to the time they approach sexual maturity, (2) that, once the infection becomes established in adult individuals, over 90 per cent remain infected, and (3) that in no herd under observation was the disease eradicated, except by a program of periodic testing and removal of the positive animals.

The earlier control experiments were limited to the University of Connecticut herd and a small selected group of private herds. In 1925 the work was extended to include several additional herds. During the past 15 years the number of herds tested annually has increased from 27 to 624, the laboratory having become the official State Laboratory for Bang's disease testing in 1931. As might be expected, expansion of the work has brought out new problems.

In recent years the experimental part of the Bang's disease project has been concerned with increasing still further the efficiency of the agglutination test, establishing the true significance of negative, suspicious, and positive reactions in different herds, determining the number of tests required to eradicate infection, determining the sources and extent of reinfection in negative herds, establishing the presence of infection in animals other than cattle, and seeking the cause of occasional abortions in Bang's disease-free herds.

### THE AGGLUTINATION TEST

The agglutination test has proved to be a strong weapon against this disease. When properly carried out it detects infection in individuals in a herd with a high degree of accuracy.<sup>2</sup> While abortion is a common symptom

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<sup>1</sup> Published in Storrs Agricultural Experiment Station Bulletins 93, 103, 108, 112, 123, 125, 130, 135, 137, 153, 154 and 185.

<sup>2</sup> Attested to by the system of retesting the same animals repeatedly, and by the close correlation of the results of the agglutination test and of the complement fixation tests conducted on the same animals.

of Bang's disease, some infected cows do not abort and some of those which do may eventually produce full-term calves. Moreover, abortions occasionally result from causes other than Bang's disease. These facts make the agglutination method all the more important in the diagnosis of Bang's disease.

A significant factor that must be kept in mind in the practical use of the agglutination test is that a period of from one to six weeks, and occasionally longer, is required between the time *Brucella abortus* enters the body of a newly infected animal and the time the host becomes positive to the test. Therefore a negative reaction given by an animal in a herd undergoing increasing infection should not be regarded as conclusive evidence that the animal is free from infection at the time.

Convincing evidence that a positive agglutination reaction is a reliable indication of infection with *Br. abortus* is found in the fact that herds from which reacting animals were removed have remained free from positive reactors in repeated tests over periods as long as 13 years. If false positive reactors had been at all common these observations would not have been possible. Furthermore, repeated tests on the same positive adult animal over periods of years have shown them to be consistently positive, except for an occasional reactor that returns to negative.

The belief that an infected animal in advanced gestation becomes temporarily negative to the agglutination test seems to be without foundation, as has been shown by repeated tests on positive animals. However, from a practical standpoint there is reason to doubt the value of a negative test on animals near parturition time in known infected or untested herds. One reason is that animals in the later stages of gestation are particularly susceptible to infection and may become infected at this time and calve between the time infection occurs and the time the blood test becomes positive.

State and federal research workers have cooperated in the past few years in improving and standardizing the tube agglutination test. The standard tube test, when conducted by experienced persons and when made according to the accepted standard, yields remarkably consistent results, as the following pages will show.

*Consistency of Reactions in Repeated Tests.* In the present Connecticut system, herds are frequently given a preliminary test to determine the number of replacements needed before subjecting the herd to a federal test. The second or federal test is usually made from one to five months after the first.

Of 938 animals in 31 infected herds, 206 reacted positively in the first test. In the second test 196 were positive, 6 suspicious and 4 negative. There was 95 per cent agreement. Available evidence indicates that the four animals which changed from positive to negative did so because they recovered from a light infection. Vaccination with dead or attenuated *Br. abortus* cells may also cause a temporary positive or suspicious reaction.

Of 665 animals in 19 negative herds, none reacted positively on retest; one gave a suspicious reaction and the remainder were negative. In this instance the agreement was 99.85 per cent.

Recently blood from 15 suspicious and positive animals was tested in four official laboratories. The results obtained are of interest because they confirm previous conclusions that there is little disagreement between responsible laboratories which employ the standard tube test. Ten of the 15 samples were positive in all four laboratories. The remaining five were recorded as suspicious by three of the four laboratories, while the fourth reported three samples as suspicious and two as positive.

#### THE SUSPICIOUS REACTOR<sup>3</sup>

The very nature of all serological tests, as well as allergic reactions like the tuberculin test, is such that suspicious (partial) reactions must occur. They represent stages of transition from negative to positive in which the infection has been too recent or slow in establishing itself to produce sufficient agglutinins or sensitization to give a positive reaction to the test. Furthermore, some animals receive a light infection which is destroyed by the animal's defensive mechanism and hence give a temporary suspicious reaction. The occasional animal that changes from positive to negative must likewise pass through an intermediate or suspicious stage.

The proportion of suspicious reactors under our observation which gave positive reactions in subsequent tests varied materially from herd to herd.

In herds which had been negative in previous tests and which possessed suspicious reactors later, the average number of suspicious animals that became positive on subsequent tests was 16.6 per cent. In infected herds having a slow spread of infection the proportion was 26 per cent, and in herds in which there was rapid spread 63.6 per cent became positive. The average incidence of suspicious reactions in a group of infected herds was about three times as great as in a group of herds that were negative as such in the previous test. This observation indicates that in the infected herds a majority of suspicious reactions were caused by *Br. abortus*. About half of these became negative on subsequent tests.

*Duration of Suspicious Reactions.* In general, animals giving a suspicious reaction may be expected to give a stronger reaction within 10 to 14 days, if the suspicious reaction is caused by an infection that becomes established; or a weaker reaction if the animal overcomes the infection, or if the suspicious reaction is not caused by *Br. abortus*. In rare instances an animal may give a suspicious reaction over a period of years. Three such animals have been observed by us.

<sup>3</sup> On the basis of extensive studies by numerous experiment stations and the Bureau of Animal Industry, a suspicious reaction may be defined as one in which agglutination is partial or complete in the 1 to 50 dilution of serum and negative or partial in the 1 to 100 dilution.

SYNOPSIS OF SEROLOGICAL TESTS FOR BANG'S DISEASE CONDUCTED AT THE  
STORRS LABORATORY

From July 1, 1925, to July 1, 1938, the number of samples tested annually increased from 2,373 to 42,700; the number of individual animals from 992 to 16,539; the number of herds from 27 to 624, and the number of herds passing one or more negative tests from 4 to 473. The number of herds passing two or more clean successive tests during the past fiscal year was 285.

The average number of animals giving positive reactions in their initial tests during the period 1925-1938 constituted 18.4 per cent for all herds, and 22.6 per cent for 686 herds which contained one or more positive animals at the time of the first test. When half of the suspiciously reacting animals are included, the results indicate that in Connecticut about 25 per cent of the animals in herds tested for the first time are infected.

PROBLEMS IN ERADICATION

In the early work on Bang's disease at the Storrs station attempts were made to control the disease in the college herd by sanitation and segregation of positive animals, and gradually replacing them with Brucella-free individuals. Success attended these efforts for about three years, at the end of which several heifers reacted to the blood test and a new wave of Brucella infection ran through the barn. On the basis of this observation and of results obtained in other herds, the conclusion was reached that segregation of positive animals in the same barn or on the same premises, or on different premises having the same personnel, is usually inadequate to prevent further infection, and that early and complete removal of all reactors is an absolute essential. Through cooperation with state and federal authorities, this policy has been adopted and is now carried out in Bang's disease control work on an increasingly large scale.

*Number of Tests Required for Eradication.* In a large majority of herds under observation the number of tests necessary to eradicate infection under the plan which requires immediate disposal of positive reactors was surprisingly small, as the following figures on 149 different herds show.

Herds requiring from 1 to 2 tests—	64.5%
“ “ “ 3 to 4 “ —	22.7%
“ “ “ more than 4 “ —	12.7%

About 13 per cent of the herds that were found to be infected in the initial tests required more than the usual number of tests. In these the rate of spread of infection was rapid. Indeed, experience has shown that eradication is accomplished much more rapidly after, rather than during, the height of a so-called "abortion storm."

Results obtained during the past few years show that in the majority of

infected herds tests made at frequent intervals (30 days between tests) are more effective in eradicating Bang's disease than tests made at longer intervals; and that the incidence of initial infection in any given herd does not necessarily bear any definite relation to the number of tests required to eradicate the infection.

*Possible Ways of Spread.* Complete solution of the problem of preventing the spread of infection requires further knowledge of the ways in which *Br. abortus* leaves the body of an infected animal, and by which it gains entrance and establishes itself in hitherto uninfected individuals.

The organism may leave the body of an infected cow in the milk and in the discharge from the genital tract especially following calving. The placenta and fetus appear to be particularly dangerous sources of infection. Whether in the milk or uterine discharges, the infectious organism may pass to other animals in one or more of the following ways: By direct contact with the infected cow, afterbirth or fetus; through contamination of the floor and eventually the feed and water; by the infectious material becoming lodged on the tail and brushed across the face, especially the eyes; by the use of bulls on infected and noninfected heifers and cows; by being carried to the feed manger on the shoes of persons who have entered isolation stalls which house infected cows at calving time; and by being carried to the teats of cows on the hands of milkers or on the teat cups of the milking machine, and then gaining entrance through the teat canal or injured skin of the teat.

The following sanitary measures should aid materially in preventing the spread of the disease in an infected herd:

1. Prompt isolation of pregnant cows at the first indication of parturition, and of all cows having an abnormal discharge from the genital tract. Calving animals should be kept in isolation until they have passed a negative test after calving. Pans of disinfectant should be placed at the entrance of the stalls occupied by isolated cows, and used freely by attendants.
2. Daily washing of the flanks, vulva and tail with a strong soap solution, followed by treatment with a disinfectant solution such as 1 per cent Liquor Cresolis Compositus or a 0.5 per cent solution of Phenolor (Squibb).
3. The rinsing of milkers' hands or teat cups of the milking machine with hypochlorite solution, containing about 300 parts per million of active chlorine, between individual milkings.
4. Blood testing at intervals of 30 days.
5. Immediate removal of both suspicious and positive reactors.

#### MAINTENANCE OF NEGATIVE HERDS

Eradication of Bang's disease from a given herd is only part of an effective control program. Once eradication has been accomplished the herd must be protected against new infection.

As long as infected herds exist in a given community there is a possibility

of reinfection from contact with infected animals on adjoining farms as the result of members of either herd becoming unconfined. However, the danger of reinfection from outside sources may be reduced to a very low minimum if owners will familiarize themselves with the basic principles of the agglutination test, ways in which new infection may gain entrance, and the present official system of eradication and control.

The incidence and possible circumstances attending reinfection in herds which have passed several negative tests have been made objects of special study in herds which have been under observation for periods of from 2 to 13 years.

*Incidence of Infection in Reinfected Herds.* Of 218 herds under observation for periods of from 2 to 13 years following initial negative tests, 67 became reinfected. In 26, or 38.8 per cent of the reinfected herds, the number of new reactors was only one; in 52, or 77.6 per cent, the number was five or less, and in 9, or 13.5 per cent, it was 10 or more. When we consider the high percentage of infected herds in Connecticut and the fact that during all of these years the control work was conducted on a more or less experimental basis, the number of "breaks" was not at all discouraging. Furthermore, new infection was limited to one or two animals in about half of the reinfected herds which were tested at regular intervals of six months.

*Number of Tests Required to Eradicate New Infection.* Of 65 herds which had one or more new reactors, 29 were found to be entirely negative to the first test following detection and removal of the newly infected animal or animals; nine additional herds were negative in the second herd test, seven in the third, seven in the fourth, and two in the fifth test. Eight herds required from six to ten tests, and three more than ten.

*Probable Sources of Reinfection.* An effort was made to determine the probable source of reinfection in 39 herds which had been negative to the blood test. Information obtained indicated that the most common cause of reinfection was association with infected cattle, as, for example, new additions to the herd and neighbors' animals with which contact was made through inadequate or broken pasture barriers.

The majority of additions that were responsible for reinfection were recently infected cows which came from untested or infected herds and which were negative at the time of purchase.

In two herds bulls which had been negative when brought in subsequently reacted positively, along with cows which they had served. In another herd a positive bull was bred to negative cows, which subsequently reacted to the blood test, as did several others in the herd.

Two herds were reinfected after a cow in estrus had broken through a barrier and mingled with infected cattle, and two were reinfected after cows from outside herds had broken into the enclosure of negative cattle.

Pasturing heifers from negative sources with those from untested herds accounted for two "breaks."

Available evidence indicated that two "breaks" were caused by association with infected horses, and one by the presence of infected swine on the premises.

It appeared that in 11 instances infection was carried into the dairy barn by persons coming directly from infected herds.

No information was obtained on eight herds.

#### BR. ABORTUS INFECTION IN ANIMALS OTHER THAN CATTLE

Nine of 104 blood samples from swine and 13 of 100 samples from horses reacted positively to the test. All of 162 samples from 20 herds of goats, two from deer, two from cats, and one from a dog were negative to the agglutination test for infection with *Br. abortus*.

#### ABORTIONS IN BANG'S DISEASE-FREE HERDS

Occasional abortions are known to occur in herds which have passed several negative tests for Bang's disease. Under such conditions the question naturally arises whether the abortion resulted from Bang's disease or some other cause. Since 1932 bacteriological examinations have been made on fetuses and placental material from 36 aborting cows in 24 herds which were free from Bang's disease, as indicated by two or more previous herd tests. The suspected material was examined for the presence of *Br. abortus* by cultural methods and by guinea pig inoculation. Bacto Tryptose agar prepared with 1 to 700,000 gentian violet, liver infusion agar, and blood agar plates were inoculated with the suspected material. The inoculated media were incubated at 37° C. in an atmosphere which contained 5 per cent carbon dioxide. The plates were examined after 3 and 5 days. Cultures were prepared from Brucella-like colonies and examined for morphological and cultural characteristics and for evidence of a serological relation to *Br. abortus*. Isolations for study were also made from other types of colonies which occurred either in significant numbers or resembled those of known pathogenic bacteria.

*Br. abortus* was not obtained from any of the 36 specimens from herds which had previously passed two or more negative tests, and the herds involved were free from positive reactors on subsequent tests.

Bacteriological tests made on a fetus from an animal in a herd which had passed only one negative herd test yielded *Br. abortus*. In this instance the cow which aborted gave a negative blood test two weeks previous to the abortion and a positive reaction when tested one week after the abortion. The results show that the aborting animal was recently infected, and that the abortion occurred during the period between the time of infection and the development of a positive reaction. The herd in question is a good example of the importance of prompt isolation of animals which abort and of not

returning such animals to the herd until they pass a negative blood test made from one to two weeks after parturition.

Bacteriological tests on the 36 specimens of fetuses and placental material in which *Br. abortus* was not present revealed streptococci distinct from *Streptococcus agalactiae* in 8 specimens; diphtheroids, the majority of which were identified as *Corynebacterium pyogenes*, were obtained from 8, hemolytic coagulase positive staphylococci from 6, *Pseudomonas pyocyanea* from 1, a monilia-like form from 1, and coliform organisms from 2. No bacteria were obtained from 10 fetuses.

Some recent observations indicate that the diphtheroids and staphylococci obtained from aborted fetuses in Bang's disease-free herds are identical with similar organisms obtained from pus from the uteri of several cows affected with metritis. Consequently, it appears that uterine infection with bovine pyogenic bacteria may result in either sterility as a result of severe metritis or, if pregnancy occurs, in abortion. Abortion associated with pyogenic bacteria is not limited to Bang's disease-free herds. The availability of herds free from Bang's disease greatly facilitates studies on bovine genital diseases due to causes other than *Br. abortus*.

#### SUMMARY

During the period 1925-1939, the number of cows' blood samples tested annually increased from 2,373 to 42,700; the number of herds from 27 to 624; and the number of herds passing one or more negative tests from 4 to 473.

The average incidence of infection observed in infected herds at the time of the initial tests was found to be about 25 per cent.

The average number of suspicious reactors that became positive was 16.6 per cent in herds which were negative in the preceding test; 26 per cent in herds in which spread of infection was slow; and 63.6 per cent in herds in which spread of infection was rapid.

Of 149 infected herds, 64.5 per cent required from one to two tests for eradication; 22.7 per cent required from three to four, and 12.7 per cent, more than four tests.

The principal source of reinfection was found to be association with infected animals, either as the result of adding cows or bulls from untested or infected herds, or of animals breaking into or out of pasture. Association with infected horses or swine accounted for several "breaks," and in some instances available evidence indicated that infection resulted when people went directly from infected to negative herds.

Bacteriological examinations of fetuses and placental material obtained from 36 aborting cows in 24 herds which were free from Bang's disease, as shown by the standard tube agglutination test, were all negative for *Brucella abortus*.

In general, available information indicated that "breaks" are preventable. In about half of the reinfected herds, regular six-month retests detected new infection before it had a chance to spread to more than one or two animals.

At the present time many negative herds exist on farms which adjoin farms on which there are untested or known infected herds. As the eradication program progresses and sources of new infection are eliminated the number of "breaks" from these sources may be expected to decrease materially.



# THE VITAMIN A REQUIREMENTS OF DAIRY COWS FOR THE PRODUCTION OF BUTTERFAT OF HIGH VITAMIN A VALUE. I. ARTIFICIALLY DRIED ALFALFA HAY (CAROTENE)<sup>1</sup>

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Numerous investigations (1-8) have shown that the vitamin A potency of milk fat is dependent upon the diet fed the cow. Several investigators (9-13) have also studied the vitamin A requirements of cattle with respect to the well being of the animal itself. Comparatively few investigations, however, have been conducted to determine the vitamin A requirements of dairy cattle for the production of milk fat of high vitamin A activity. Fraps, Copeland and Treichler (14) observed that when lactating cows were restricted to 17,000 vitamin A units daily from yellow corn the vitamin A potency of the milk fat decreased from 38 to 16 units in four weeks. These workers also found that feeding 116,000 vitamin A units daily failed to maintain the vitamin A potency of the milk fat. Later the same investigators (15) found that 340,000 daily unit intake failed to maintain the vitamin A potency of the butterfat and estimated that from 750,000 to 1,400,000 Sherman-Munsel units were needed daily by cows to produce butterfat of high vitamin A value. Atkeson and associates (16) concluded that butterfat secreted by dairy cows fed a ration containing 1,000,000 international units of carotene daily was typical of grass produced butter. Increasing the carotene intake to approximately 6,000,000 units daily resulted in practically no change in the carotene and vitamin A content of the butter.

## EXPERIMENTAL

The general plan of procedure in these experiments has been to decrease the vitamin A potency of the milk fat to a low level by feeding the cows vitamin A deficient diets, and then determine the number of vitamin A units required daily by the cows to bring the vitamin potency of the milk fat back to a high level. The vitamin A deficient ration was composed of beet pulp, and a grain mixture of white corn, oats and linseed oil meal. The principal source of vitamin A in the repletion rations was artificially dried alfalfa hay. Two separate experiments similar in nature have been completed.

## EXPERIMENT I

Two Guernsey cows were used in this feeding experiment. They were

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<sup>1</sup> Published with the approval of the Director of the Purdue University Agricultural Experiment Station.

in the early stage of lactation at the beginning of the feeding trials and continued in normal milk flow throughout the experiment. The cows were fed the vitamin A deficient diet until the vitamin A potency of the milk fat had dropped to a low level (12 units). At this point, one, two, three, five, eight and twelve pounds of artificially dried alfalfa hay of known vitamin A value were added to the ration in successive 21 day feeding trials. Twenty-one

TABLE 1

*Showing the vitamin A requirements of dairy cows when dehydrated alfalfa hay (carotene) was the source of vitamin A activity of the ration (1935-1936)*

Period No.	Vitamin A supplement*		Vitamin A butter (units per gram)
	A.D. alfalfa hay (lb. daily)	Unit intake (000)	
1 .....	None	None	12
2 .....	1.0	75	12
3 .....	2.0	150	12
4 .....	3.0	225	11
5 .....	5.0	375	19
6 .....	8.0	600	31
7 .....	12.0	900	30

\* Ration consisted of beet pulp and a grain mixture consisting of 400 lb. white corn, 200 lb. oats, and 150 lb. linseed oil meal.

day feeding trials were used because previous studies (17) had shown that the major change in the vitamin A activity of milk fat resulting from a change in diet takes place during the first fifteen days.

Representative samples of milk were collected from each cow during the last four milkings of each period, the cream separated and churned into butter. Each sample of butter was then subjected to biological assays for vitamin A potency. The results of these assays are expressed in Sherman and Munsell vitamin A units (18).

TABLE 2

*Showing the vitamin A requirements of dairy cows when dehydrated alfalfa hay (carotene) was the source of vitamin A activity of the ration (1936-1937)*

Period No.	Vitamin A supplement		Vitamin A butter (units per gram)
	A.D. alfalfa hay (lb. daily)	Unit intake (000)	
1* .....	(check)	Not determined	33
2† .....	None	None	14
3† .....	1.0	67	15
4† .....	3.0	201	15
5† .....	5.0	355	25
6† .....	8.0	536	29
7† .....	12.0	804	31

\* Ration consisted of alfalfa hay, silage and a grain mixture consisting of 400 lb. yellow corn, 200 lb. oats, and 100 lb. linseed oil meal.

† Ration consisted of beet pulp and a grain mixture consisting of 400 lb. white corn, 200 lb. oats, and 100 lb. linseed oil meal.

## EXPERIMENT II

Two Guernsey cows were used in the feeding experiments of the second trial which was essentially a duplication of the first trial. These cows were also in the early stage of lactation at the beginning of the experiments and continued in normal production throughout the tests.

The feeding schedule and the biological assays of the hays and butterfat for the two experiments are shown in tables 1 and 2 and figure 1.

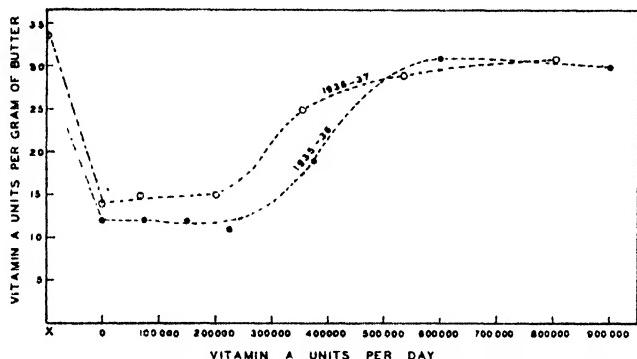


FIG. 1. The vitamin A potency of the butters produced by cows when fed different levels of vitamin A (dehydrated alfalfa hay).

## DISCUSSION

In these experiments, the criterion for the measurement of the vitamin A requirements of dairy cows for the secretion of milk fat with maximum vitamin A value is based upon the supposition that cows are not able to secrete butterfat of maximum vitamin A value until the optimum requirements for maintenance and production have been satisfied. Since the vitamin A value of butterfat secreted by the cow is dependent on the ration fed the cow, it is apparent that whenever cows secrete butterfat of low vitamin A value, this is indication of an inadequate supply of available vitamin A in the ration. Furthermore, if more potent butterfat is produced upon increasing the vitamin A intake, this would also indicate that the vitamin supply had been inadequate. Only when further additions to the rations give no further response in the potency of the butter, is there any assurance that a point of saturation has been reached. Thus, the minimum vitamin A potency of the ration which will produce the maximum effect upon the milk fat secreted should prove to be the minimum vitamin A requirement of the cow for the secretion of milk fat of high vitamin A value.

As shown in tables 1 and 2 and in figure 1, the vitamin A value of the milk fat secreted by the cows was reduced to relatively low levels by feeding a ration deficient in vitamin A. In successive feeding periods, the addition of various amounts of vitamin A (carotene) up to 225,000 units per day

did not result in any appreciable repletion but was capable only of maintaining a vitamin A level equivalent to that of the preliminary depletion period. Not until a daily intake of over 300,000 units was introduced into the ration was a significant increase apparent in vitamin A potency of the milk fat. This repletion continued with successive increases of vitamin A in the ration until a saturation was reached at approximately 550,000 units per day as evidenced by the restoration of the high potency of the milk fat. Additional vitamin A units in the ration failed to produce a significant increase in the milk fat. In these studies the vitamin A requirements of the cows to produce milk of high vitamin A value is somewhat less than that suggested by Fraps and co-workers (15).

In these experiments, the source of vitamin A was the carotene in artificially dried alfalfa hay. The alfalfa was harvested in the tenth-bloom stage and was of excellent quality. The vitamin A values of these hays were determined by biological assays. Although there was some difference in the vitamin values of the hays, there was a close correlation between the vitamin A intake and the vitamin A value of milk fat secreted in the two experiments. This would indicate that the utilization of the carotene in the hays of these experiments was approximately the same. However, it is to be recognized that the carotenes in various feeds or even vitamin A per se, may be utilized with different degrees of efficiency. The type of hay, the fertility of the soil, the maturity of the plants, the methods of preservation may influence the availability of the vitamin.

#### SUMMARY

1. Two feeding experiments have been completed to determine the minimum vitamin A requirements of dairy cows for the production of butter with maximum vitamin A value.
2. Artificially dried alfalfa hay was used as the source of vitamin A (carotene).
3. Under the conditions of these experiments, it was found that dairy cows required approximately 550,000 vitamin A units daily to restore the vitamin A potency of the milk fat to its highest value.

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## THE THIRTY-FIFTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ  
*Secretary-Treasurer*

The American Dairy Science Association was called to order by President E. S. Guthrie, in the Purdue Memorial Union Building at Purdue University, on Tuesday, June 25, at 9:30 A.M. for the thirty-fifth annual meeting.

The program printed in the June issue of the JOURNAL OF DAIRY SCIENCE was prepared by the Program Committee. The June issue also contains the abstracts of the papers presented.

Dr. E. C. Elliott, President of Purdue University, was introduced by Past President H. W. Gregory, and delivered the address of welcome. President Guthrie then gave the following response:

"We thank you, President Elliott, for your cordial welcome. Already we are enjoying the happy spirit of hospitality of your campus. The men and women, who have charge of the buildings, seem to know exactly how to make us comfortable. We have known your dairy staff as enthusiastic colleagues in dairy science. We are now recognizing in them the ideal qualities of hosts, and we admire the splendid assistance of their wives.

"This is the second year that our Association has held its annual convention in Indiana. The first one was in Indianapolis at the time of the National Dairy Show in 1925. We are happy to return,—this time to enjoy the fine accommodations of your facilities here at Purdue University."

President Guthrie then introduced Dr. Paul F. Sharp, of Cornell University, who responded by a most interesting discussion of the milk fat globule.

### THE MILK FAT GLOBULE

PAUL F. SHARP  
*Cornell University, Ithaca, New York*

President Guthrie, President Elliott, members of the American Dairy Science Association, and ladies:

I have a very small but to me a very interesting subject. The fat in milk is present in the form of little spheres about one ten-thousandth of an inch in diameter. The small size of the subject does not preclude, however, the use of large, perhaps incomprehensible numbers. In the beginning I wish to tell you that I am full of my subject as a speaker should be, having had 15 hundred billion fat globules for breakfast. In other words, I drank a glass of milk. If fat globules were dollars, 12 cc. would pay the national debt as it stood two weeks ago. Women can count money faster than men and it is estimated that a woman can count one million one dollar bills in a

month. If the fat globules were dollar bills it would take 1000 women 321 years to count the fat globules in a quart of milk. There are more fat globules in 1 cc. of milk than there are people living on the earth today. Cows in the United States produce each year  $1.67 \times 10^{23}$  fat globules. The Department of Agriculture and Dr. Campbell have not yet undertaken the task of supplying data on milk produced by other sources. If all of the fat in the globules of cows milk produced each year in the United States were brought together in one mass it would yield a cube 137 yards each way. Small as the fat globules are, and tremendous as are their numbers, yet to visualize relative magnitudes, it is interesting to note that there are as many atoms in 3.3 grams of carbon as there are fat globules produced in cows milk in the United States each year.

If the fat globules in one quart of Guernsey milk were laid end to end they would form an invisible thread of beads 5860 miles long. The thread would be 20 miles shorter if Holstein milk were used.

The fat globules in a quart of Guernsey milk have a total surface of 1050 square feet, and of Holstein milk 880 square feet. A quart of milk contains about 40 cc. of fat. If one were required to paint the surfaces of the fat globules in one quart of milk, one gallon of paint would not be sufficient. The cow is, however, a much more efficient painter; she coats the surface of these fat globules and uses only 0.25 gram of material, or an amount equivalent to about 5 drops of the kind of coating the cow puts on them.

It may be of interest to students of milk secretion that on the average a quart of Guernsey milk contains about the same number of fat globules as does a quart of Holstein milk. This indicates that the milks differ not so much in number of fat globules secreted as in their size.

#### *Composition of the fat*

Milk fat is rather unique among natural fats in that the glycerides present are esters formed from such a variety of fatty acids. Milk fat contains from 8 to 14 different fatty acids fastened to glycerine in various possible combinations of one, two or three acids. Working out the various combinations of three acid molecules fastened to one glycerine molecule, several hundred different kinds of molecules are possible in milk fat. No one knows how many different kinds of molecules are actually present. The average fat globule contains 10 billion molecules of fat.

The melting point of the fat is often reported as a characteristic physical property, and it is defined as the temperature at which the last crystal of fat disappears upon warming. Actually, the rate of warming exerts some influence upon this melting point. However, the definition would also hold for the formation of a solution. If we took a mixture of different crystalline sugars and water and warmed the mixture, we would find a temperature at which the last crystal of some one of the sugars would disappear. Ordin-

narily, this would not be a true melting point but would be called the temperature of solution or perhaps of saturation. Often it is not appreciated that the so-called melting point of milk fat is similar to the solution of the hypothetical mixture of sugars. Fat fractions can be separated from milk fat which do not melt until a temperature of 60–70° C. is attained, while these same materials when present in their normal milk fat environment disappear as solid phase when a temperature of 35° C. is reached. Also fractions can be separated which do not crystallize after holding for months at 0° C. Thus this so-called melting point of milk fat is the temperature at which the most insoluble fat fraction dissolves in the solution of glycerides.

Many factors affect the relative proportions of fatty acids present in milk fat. The most commonly mentioned factors are the feeding of oil products which tend to increase the proportion of oleic acid and yield a softer milk fat. Dry feeds are more common in winter than in summer and less oleic acid is usually present in winter fat than in summer fat. Starvation has been shown to increase greatly the oleic acid content of milk fat.

The so-called color of milk fat is of considerable commercial importance. The color is not due to colored glycerides but to the presence of carotene dissolved in the fat. Carotene is the precursor of vitamin A and from the nutritional standpoint its presence or the presence of vitamin A in the fat is very desirable. In general, yellow market milk is preferred. It has a sales appeal because yellow color has been associated with high fat content. The yellow color of whole milk is due to three factors—the amount of carotene pigment in the fat globules, the size of the fat globules and the amount of riboflavin in the water phase. With the same amount of carotene in the fat and the same amount of fat in the milk, milk containing the large fat globules will appear more yellow to the eye than milk containing small fat globules. Definite statistical breed differences in the color of milk fat have been demonstrated; the Guernsey milk fat tends to contain more carotene than the Holstein milk fat whereas, Holstein milk fat contains more pre-formed colorless vitamin A. However, no milk fat would be colored yellow unless carotene were supplied to the cow in the feed.

Under one set of conditions, at least, the yellow color in the milk fat is considered objectionable. Consumers seem to prefer a butter of average yellow color. If the yellow color is too intense, as occurs particularly in butter made from Jersey and Guernsey milk when the cows are on rich spring and summer pasture, consumers object because they think the butter is artificially colored. In certain markets sale of this butter is difficult and ways of decolorizing it or of destroying the carotene have actually been considered.

#### *Physical state of the fat*

The great variety of fat molecules present in the globules, as well as many other related observations, indicate the desirability of specific knowledge as

to the physical state of the fat in the fat globules under a variety of conditions. Information as to the physical state of the fat when the temperature of pure fat in a beaker is altered does not give this desired information, for the reason that great lags occur in the adjustment of the physical state of the fat to a new environmental condition. For example, if liquid fat is being cooled the presence of a few crystals in the beaker by seeding induces the crystallization of fat throughout the whole beaker. When fat is present as fat globules the appearance of a crystal in one fat globule exerts no seeding effect on the fat in any other globule. Consequently the lag in adjustment of the physical state of the fat when suspended as fat globules is very much greater than the lag in adjustment of the physical state of the fat when present in a beaker.

The information as to the actual physical state of the fat in the globules must be obtained by indirect methods. The two most successful methods are change in specific heat and change in specific volume. Studies of this type indicate that at least four hours must pass before cooled fat even approaches an equilibrium state. The experiments also indicate that it may take months for the final adjustment to be reached. Within the solidifying zone it is not sufficient to state the temperatures of milk and cream in order to define their properties. The previous temperature-time history through which these products have passed prior to reaching the temperature exerts a profound influence on the properties of the products. A few of the numerous illustrations of this effect will be mentioned.

#### *Lactometer readings*

The density or lactometer reading of milk used in conjunction with the fat content for the calculation of total solids is carried out at a standard temperature of 60° F. (15° C.). It may make a difference of 1 or more lactometer degrees (0.001 in specific gravity) whether the milk was cold milk warmed to 60° or heated milk cooled to 60°. Because the lag in adjustment of the physical state of the fat is greatest near 60° F. a more unsatisfactory temperature for making this density reading could not have been found. If the milk is previously warmed to about 45° C. (113° F.) and then cooled to 30° C. and the density determinations made at 30° C. (86° F.) the fat will be present in only one physical state—the liquid state. We are only certain of the density of fat when it is in the liquid state.

#### *Centrifugal cream separation*

Aside from the mechanical construction of the separator and the rate of flow of milk through it, the properties of the milk affect the movement of the fat globules through the plasma phase under centrifugal force. These properties are: first, the viscosity which decreases as the temperature increases; second, the difference in density between the fat globules and the plasma phase which increases with temperature; and third, the size of the fat glob-

ules which increases on warming and decreases on cooling. These factors are not linear functions of temperature. The relationship of these factors is such that increasing the temperature in the low temperature range—that is, between 0 and 25–30° C.—increased the effective force of separation by about 33 per cent for each 5° C. increase in temperature. In the range from 40–45° C. on up, the effective force is increased by only about 10 per cent. Thus, there is a sharp bend in the curve indicating that warming is markedly beneficial in the separation of milk up to a temperature in the neighborhood of 40° C. or 100° F.

In late years, there has been a tendency to use lower and lower temperatures of separation, because more viscous cream is obtained. The better body probably results from the absorption of the agglutinin on the fat globules in the low temperature range. The fat lost in the skimmilk, when milk is separated at an intermediate temperature, in the neighborhood of 70–80° F., is influenced by the previous temperature history of the milk. The fat loss in the skimmilk is less on milk which was previously warmed and cooled to this temperature for separation because the fat will be in a more liquid state, thus maintaining a greater difference in density between the fat and the plasma and because the fat globules in the liquid state are larger than the fat globules in the solid state. The conditions would be reversed in the case of cold milk warmed to the same temperature for separation.

Another interesting observation relating to temperature of separation was found as a result of preheating milk to a series of temperatures previous to separation at 100° F. Samples of milk were pasteurized for 30 minutes at temperatures up to 176° F. (80° C.). These samples were then cooled to 100° F. and the milk was separated immediately. The fat content of the cream increased progressively as the temperature of pasteurization increased.

### *Churning*

The physical state of the fat exerts a profound influence upon churning. Normal churning occurs only when the fat in the fat globules is in the part solid, part liquid state. At low temperatures (50° F. or below) the fat is essentially solid and churning does not occur. At temperatures above 100° F. the fat is almost completely liquid and again churning does not occur. The churning time between these two temperature zones depends upon the relative proportions of solid and liquid fat and whether or not liquid fat globules are cooled into the churning zone or solid fat globules are warmed into the churning zone. The shortest churning time is not obtained at the same temperature when approached from these two different directions. It occurs at lower temperatures for the previously liquid fat and at higher temperatures for the previously solid fat. The properties of the butter and the fat lost in the buttermilk are also influenced by the proportion of solid and liquid fat at churning time.

Incidentally, the presence of casein in the cream exerts a profound influence on the churning time. If the casein is in a precipitated state, as in sour cream or cream to which rennet has been added, the churning time is relatively short as compared with that for cream in which the casein is hydrated and highly dispersed. Another interesting point is shown by the effect of acidity on the churning of washed cream. Within the practical range, the more acid the normal cream the shorter the churning time, but in the case of washed cream, that is cream in which the plasma phase has been replaced by water and thus casein is absent and only the materials on the surface of the fat globules remain; the more acid the washed cream down to pH about 4.3, the longer the churning time.

### *Lipolysis*

Ordinarily, we consider that the increase in acidity of milk and cream is due to the production of acids by the growth of bacteria, but under certain conditions the acidity of the milk and cream increases definitely in the absence of any appreciable number of bacteria. For a while it was thought that this increase was due to the action of bacteria which had not been detected in the milk, but it is now generally accepted that this increase in acidity is a result of hydrolysis of the milk fat by enzymes with the liberation of the free fatty acids, and it is these free fatty acids which cause the increase in acidity. In raw cream held below 40° F. this increase in acidity may amount to 0.05 per cent expressed as lactic acid, in 24 hours. In addition to this difference, detected chemically by the increase in acidity, the presence of the free fatty acids gives to milk and cream an undesirable odor and taste, often described as bitter, butyric acid or rancid.

Much work has been done on the conditions affecting the lipolysis of milk fat. It has been found that if cream is to be separated from milk, particularly to produce viscous cream in winter, it is advisable to warm the milk to about 120° F., then cool it to the separation temperature of about 70–80° F. When this is done, the cream shows relatively little lipolytic activity as compared with cream separated from cold milk warmed to the separation temperature. Tests show that lipase is present in both creams but in one it is inactive. This result indicates that the previous temperature treatment of the milk has a profound effect on lipolytic action in the cream separated from it. It has been shown very clearly that when cold milk is warmed to 30° C. and then cooled to some low temperature, the lipolytic action is very much greater when compared with milk which has not been subjected to this cooling, warming and cooling or has been cooled and warmed to any higher or lower temperature. The term "temperature activation" has been applied to this process.

It has been shown that the normal fat globules of milk or cream show greater lipolytic activity the lower the temperature of holding. This is particularly true in regard to cold milk heated to 30° C. and recooled to a series

of temperatures. Thus, fat globules with natural surfaces show a negative temperature coefficient of lipase action. In other words, the lower the temperature the greater the increase in activity. This is contrary to the expected effect of temperature on enzyme action, and is the only case of a negative temperature coefficient of enzyme action of which I am aware. This negative temperature coefficient is confined to fat globules having the natural surfaces. If milk fat is reemulsified in skimmilk to produce milks or creams containing fat globules of approximately normal size, the temperature coefficient of lipase action is positive—that is, the higher the temperature the greater the lipolytic action. Furthermore, such resurfaced fat globules show no additional activation effect upon warming and cooling. The mere resurfacing of the fat globules serves as an activation mechanism. The fat does not need to be removed from the milk in order to resurface and activate the lipase, but it can be resurfaced and activated by homogenizing the milk or cream.

The concentration of substrate, that is, the percentage of fat in the milk or cream has been found to influence the character of lipase action. When raw products containing increasing amounts of fat were temperature activated, it was discovered that with an increase in fat up to about 6 to 8 per cent, the water phase increased in titratable acidity up to about 0.05 per cent expressed as lactic acid, and that no further increase in acidity of the water phase occurred at higher fat contents. Up to about this same fat content, the acidity of the fat per unit of fat increased, and from this point on decreased, although the titratable acidity of the total fat present increased with the increase in the amount of fat in the cream. This indicates that the lipase action on glycerides containing the short chain fatty acids which are soluble in water is stopped by the accumulation of the fatty acids in the water phase, but that the lipase continues to hydrolyze the fats of the longer chain acids—acids which do not dissolve in the water but remain in solution in the fat . . . until these also accumulate to an amount sufficient to stop the action. This points to studies on the reversibility of lipase action and the selective hydrolysis of the various glycerides of milk fat by the same enzyme system under conditions varying principally in respect to the concentration of substrate and ratio of water to fat phase.

The specific surface properties of the fat globules exert a profound effect upon the rate of lipolytic action. This was demonstrated by experiments with re-mixed milks. Skimmilks from a number of individual Jersey and Holstein cows were used. The lipase resides in the skimmilk or water phase. Two series of skimmilks, one series obtained from Jersey cows, the other from Holsteins, were used. The same cream was added to both series. We thus had two sets of samples, each set containing the same amount of the same fat and of the same kind of fat globules. We would expect the lipolytic action in the two sets of samples to be directly parallel and comparable.

This was not the case, indicating rather specific properties of the surfaces of the fat globules.

The acids liberated by lipolysis of milk fat exert an effect upon salt equilibrium, surface tension, foaming, and churning. The fatty acids liberated lower the surface tension and tend to create a stable, soap-like foam. Due to the formation of soaps in the water phase, this type of foam structure interferes seriously with churning. From the practical standpoint, the high lipolytic activity of the milk from cows in advanced lactation pronouncedly retards churning and often milk or cream in winter is pasteurized as soon as possible in order to inactivate the lipase and overcome this difficulty.

#### *Gravity creaming*

The mechanism of gravity creaming has long intrigued investigators. An early explanation was that when the milk was cooled the density of the water phase increased, while the increase in density of fat showed a lag, and it was thought that this increase in the difference in density caused gravity creaming. An idea along somewhat similar lines was that when milk was cooled the heat liberated by the solidifying fat globules caused the rising of the cream. Another explanation was a sort of a swarm theory, the idea being that the large fat globules overtook others and formed a swarm, thus creating a greater unit size, resulting in the rapid rising. It has of course been observed that Jersey milk gives deeper cream layers than Holstein milk, and that fat globules in Jersey milk are larger than the fat globules in Holstein milk. These two variables were connected to form the basis of a theory of creaming and it has been stated that one gets more cream on Jersey milk because the fat globules are larger. It is true that the fat globules are larger but you get more fat from Jersey milk because Jersey milk contains more fat and you get deeper cream lines because an agglutinating substance is present in the milk in approximately the same proportion as the fat, as experiments presented by Palmer and Dahlberg may be interpreted as showing. The difference in size of the fat globules does not explain why Holstein milk or Jersey milk for that matter creams in a few hours.

In recent years application of Stokes' law has been made to the problem of gravity creaming. Stokes' law applies to the rate of movement of small spheres through a viscous medium when moving at constant velocity, due to a gravitational force. Calculations indicate that it would take about 300 to 500 hours at 5° C. (41° F.) for an individual fat globule to move from the bottom to the top of a quart milk bottle. This indicates that gravity creaming does not result from the movement of individual fat globules. We know now that gravity creaming occurs as a result of the clumping of the fat globules into agglomerates comprising hundreds or even thousands of fat globules. These clumps rise according to Stokes' law, and the size of the agglomerates are such that creaming can occur in from one to two hours—that is, the normal gravity creaming time.

It has been found that raw milk contains a small amount of a protein material which will "agglutinate" the fat globules. The term agglutination was applied to the clumping of fat globules some years ago by the investigators at the Hoorn Experiment Station in Holland. The natural agglutinin is adsorbed on solid or solidifying fat globules and causes the globules to clump. It is liberated to the plasma and the clumps fall apart when the fat globules melt, to be readSORBED and cause reclumping upon recooling. The agglutinin is heat labile and solid fat globules do not adsorb the agglutinin after it has been heat altered. The fat globules in heated milk do not clump. The behavior of the agglutinin accounts for most of the known factors of gravity creaming. The properties of the agglutinin and the fat point to a rather interesting way of enhancing gravity cream volumes. According to this idea, agglutinin is present on the fat globules if the fat globules are separated from the milk while the fat is in the solid state, and thus would be absent from the skimmilk. If the fat globules are separated while the fat is in the liquid state, then the agglutinin would not be present on the fat globules but would remain in the skimmilk. Thus, by combining low-temperature-separated cream with high-temperature-separated skimmilk, good gravity creaming milk is obtained, while by combining low-temperature-separated skimmilk with high-temperature-separated cream, poor gravity creaming milk results. Another interesting test of this idea is obtained by separating cream to about 25-30 per cent fat content at a low temperature, and then reseparating the cream to a higher fat content at a temperature at which the fat globules are liquid. This gives a cream plasma which is very much enriched in agglutinin, and recombined milk made with this cream plasma gives very deep cream layers, sometimes yielding 80 per cent of cream on 4 per cent fat content milk. Agglutinin added to homogenized or heated milk which otherwise does not cream causes the fat to cluster and produces good gravity creaming.

#### *Cream viscosity*

Under proper conditions the presence of the natural agglutinin in cream yields cold cream of high viscosity. The agglutinin will be present in the cream if the cold milk is separated at a low temperature. It was observed a number of years ago by Dahlberg that if milk was pasteurized, cooled, and held cold for a few hours and then separated it would yield a cream of as high viscosity as that obtained from raw milk. The attempt to make the agglutinin go with the cream has resulted in lower and lower temperatures of separation of cream for market purposes.

In the last few years a procedure has been developed whereby cold cream is warmed relatively slowly to a temperature in the neighborhood of 30° C. and then is recooled relatively slowly. Such a procedure tends to increase the viscosity of the cream. A lead toward the explanation of this behavior

is to be found in the effect of such a procedure in altering the physical state of the fat. In the fat as it is originally solidified a great variety of fats crystallizes out. The fat is then warmed until nearly all of the kinds of fats dissolve, but not quite all of them. Then the fat is recooled. On this second cooling, the fat globules are cooled in the presence of seeding nuclei of the most insoluble fat fraction and consequently this fraction crystallizes out more rapidly and completely the second time that it did the first time when it was not seeded. This alters the relative proportions and the rate of crystallization of the fat fractions and yields a different solid phase on this second recooling. For some reason or other this different physical state of the fat on the second cooling has an influence upon the surface of the fat globules, perhaps through a different orientation of molecules and consequently upon the materials adsorbed, which apparently results in this increase in viscosity.

The conditioning of the fat by cooling, warming and cooling activates the lipase in raw milk, produces viscous cream as just described and is used to condition milk chocolate for candy coating. Evidence has been obtained by Rishoi that the specific heat of this recooled fat is different from that of the fat cooled the first time.

#### *Composition of natural material on the milk fat globule surface*

The identification of the materials present on the surface of natural milk fat globules has attracted investigators for years. In the various reports we find that nearly everything present in milk has been reported by some investigator to be adsorbed on the surface of fat globules. Among the early investigators was Völtz, who found lactose, ash, casein, and albumen were all adsorbed on the surface of the fat globules. His results were obtained because of faulty technique of separating the fat globules. He separated some skimmilk (plasma phase) along with the fat and consequently when he carried out his analysis he found these skimmilk components. Others have made errors of this kind in separating the fat globules. Storch concluded that the material was a protein of mucin type because it had reducing properties. He separated cream, mixed it with water, and reseparated it, repeating this procedure four times. He thus displaced the skimmilk phase with water and concluded that the protein remaining was on the surface of the fat globules. He found 2 grams protein adsorbed on 100 grams fat. Van Dam and Sirks made protein analyses on skimmilk and cream and discovered that the protein content was higher in cream when calculated to the basis of the water phase. The difference between these two analyses was attributed to the protein adsorbed on the surface of the fat. They found that 0.29 to 0.82 average 0.47 gram of protein on 100 grams of fat. Whitaker, using the washing method, concluded that about 0.31 to 0.56 gram, best value 0.35, was present on 100 grams of fat. Hattori found that a protein different from

the others in milk was present on the fat. Palmer and his co-workers washed cream a few times, churned it, and assumed that the surface material and surface material only was liberated in the buttermilk. They concluded that the protein obtained had not been identified previously. They also found phospholipid material and concluded that ether extractable non-phospholipid material comprised 50 per cent of the membrane.

Recent results indicate that the reddish color of raw cream buttermilk is due to a flavoprotein. Using a method which would differentiate between free flavin in milk and the flavoprotein, it was found that there was present on the fat globules about 0.07 gram of flavoprotein per 100 grams of fat. This flavoprotein appears to be the Schardinger enzyme or xanthine oxidase. Calculations indicate that 66,000 molecules of this enzyme material are present on the surface of each fat globule. The flavoprotein may adhere quite persistently to the surface of the fat globule, and at least half of it cannot be removed by washing.

Rimpila and Palmer show that after washing six times 50 per cent of the phosphatase activity remains with the cream. This shows phosphatase in the membrane.

In addition, it has been shown that at cold temperatures the agglutinin is adsorbed on the surface of the fat globules, but the evidence is that the agglutinin was removed in the washings in the procedures used for isolating the materials on the surface of the fat globule, and consequently the agglutinin was not present in the surface material analyzed by other workers.

Davies has shown that a copper protein compound is adsorbed on the surface of the fat globules, and that copper added to milk is present in a greater proportion on the surface of the fat globules than would be accounted for by equal distribution throughout the milk.

Sirks, as well as Sommer, has studied the electrical charge on the fat globule and the fat. Sirks could find no relation between charge on the fat globule and creaming, whereas Sommer concluded that alterations in electrical charge explain some of the differences in gravity creaming.

#### *Resurfaced fat globules*

Various attempts have been made to redisperse butterfat and recreate fat globules in milk or cream having the same properties as the naturally surfaced fat globules. On the whole, these attempts have been unsuccessful. The product will not churn properly, or it will not withstand washing with water, its reaction to lipase is altered, it will not have the Schardinger enzyme reaction, and almost invariably it will yield clear whey when the attempt is made to filter it. These products ordinarily do not give normal gravity creaming, but some of them will cream normally if agglutinin is added.

In this talk I have tried to present to you a picture of the research that

can be carried out on an object so small and so much taken for granted as the milk fat globule. I hope I have been able to convince you that research on the fat globule is worth while and has led to results which are of scientific interest and of practical value.

#### PRESIDENT'S ADDRESS

The thirty-fifth annual meeting of the American Dairy Science Association takes me back in memory to the corn-growing days of 1906, when I came to Purdue University from Ohio State University to join my friend and classmate, Fred Rasmussen.

We Iowa lads then journeyed to the University of Illinois to attend a meeting of dairy instructors and investigators which was called to convene during the first session of the Graduate School in Agriculture. This school met at different colleges of agriculture on alternate summers for several years. It was at the first meeting of this school that Professor W. J. Fraser, Chief of the Dairy Department at the University of Illinois, assembled a group of instructors and research workers on July 17, 1906.

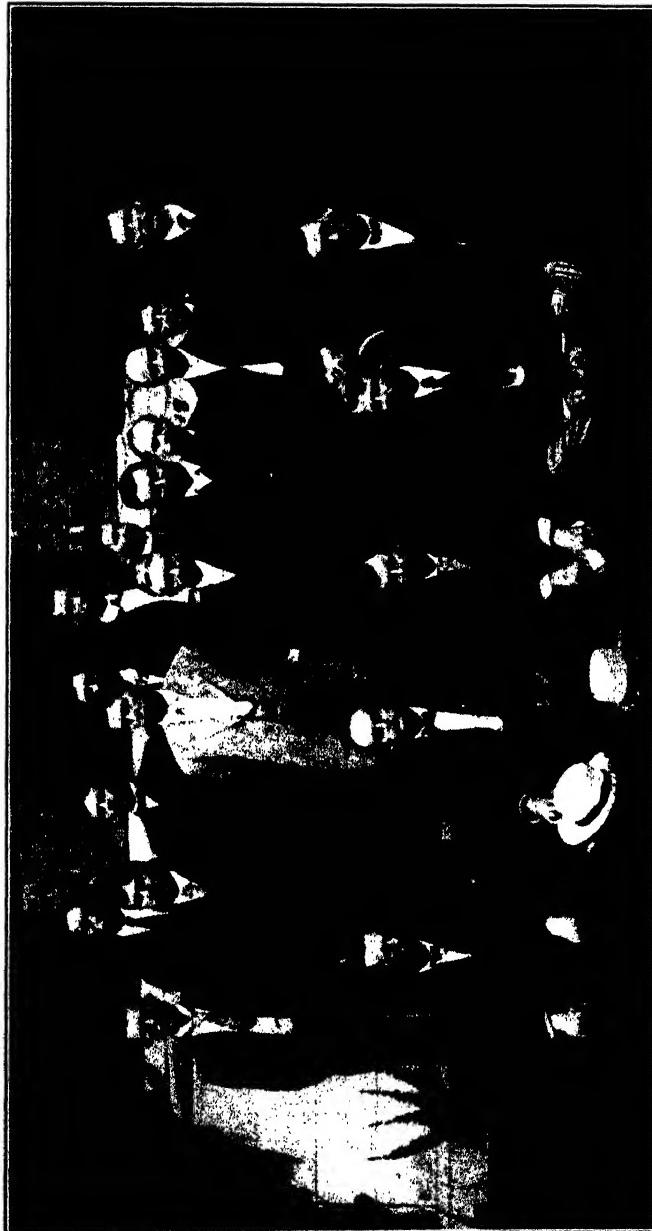
After several periods of presentation of papers and discussions, an organization was launched. Fortunately, that little group, the general appearance of which is recorded in the first picture, had in it several individuals of splendid leadership and who soon became world characters. My friend, Fred, and I were junior members of this new enterprise and I can assure you that we were impressed by the information that we obtained. Later we realized that still more important than the knowledge that we received was the friendship of the older men.

I hope that it is realized that the task of preparing a short historical sketch is not an easy one. Perhaps, however, I can continue in giving a review that will be of value to our younger members.

A brief meeting of our Association was held at the National Dairy Show in Chicago, Illinois, on October 11, 1907. The next session was in the summer of 1908 at Cornell University, where the Graduate School in Agriculture met for the second time.

The first president, the former Professor R. A. Pearson, offered a comment on a change in name in the beginning paragraph of the president's address. He stated: "The name of our organization is not descriptive of its character. We call it 'The National Association of Dairy Instructors and Investigators' yet some of our members represent the great dairy interests of Canada. It is then, strictly speaking, not a national organization. . . . I would like to suggest as a new name, "Official Dairy Instructors Association.'"

When meeting under the new name on October 24, 1910, in Chicago, Illinois, our second president, the former Doctor C. H. Eckles, voiced an expression for cooperation, which I wish to quote. Before I read his state-



FOUNDERS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION, UNIVERSITY OF ILLINOIS, 1906.

(1) C. C. Hayden, Illinois, (2) J. W. Decker, Ohio, (3) C. F. Doane, U.S.D.A., (4) W. J. Fraser, Illinois, (5) H. A. Hooper, Illinois, (6) J. M. Truman, Illinois, (7) C. H. Eekles, Missouri, (8) E. H. Webster, U.S.D.A., (9) A. C. True, U.S.D.A., (10) H. H. Dean, Ontario, (11) C. B. Lane, U.S.D.A., (12) Fred Rasmussen, Indiana, (13) C. E. Lee, Illinois, (14) O. F. Hanziker, Indiana, (15) E. S. Guthrie, Ohio, (16) H. E. Van Norman, Indiana, (17) Charles Thom, U.S.D.A., (18) Eugene Davenport, Illinois, (19) B. D. White, U.S.D.A.

ment, may I say that sometimes I think it is almost sacrilegious for a presiding officer or any one else, to offer comments on an excellent statement or address. That is my sentiment as I read this paragraph from Doctor Eckles.

"Dairy husbandry is the application of several sciences to certain practical lines and for this reason a man directing such work should have a rather broad training without being, necessarily, a specialist in any one. When a chemist alone undertakes to carry on an investigation with animals he is apt to overlook some essential points in treatment of the animals used and he is not in a position to know the problems that need solution from a practical standpoint. On the other hand one filling a responsible position along dairy lines does not have as a rule the intimate knowledge of chemistry and especially of physiological chemistry necessary to properly carry on research to good advantage. The man in this position, however, has an opportunity to know the problems that the practical man wants solved and he should combine with this the technical knowledge of the management of the animals or the manufacture of the dairy products as the case may be. The plan that must be followed in the future, if we gain much headway, is to work in groups. One of the group must have a broad general knowledge and be familiar with the practical side of the problem. Associated with him must be competent chemists, physiologists and bacteriologists, according to the problem at hand."

On October 29, 1912, in Chicago, Doctor O. F. Hunziker, our third president, when speaking of the accomplishments of our Association, made this statement:

"We have grown not only in members but also in the scope of our activities and usefulness and our results have gained recognition in state and nation. We have accomplished the standardization of Babcock testing glassware, so that today, it is possible for anyone to secure standard glassware that is correct, accurate and uniform so that the results of its use are comparable. We have been instrumental in the unification and perfection of a national score card for scoring dairies, in the purification of national judging contests and in the modification of the national standards for milk products.

"We have discussed and analyzed, for the benefit and information of all, the principles and methods of instruction in dairying, our relation to the breed associations, experimental work in milk production and dairy manufactures, proper standards for dairy products, standard methods of testing milk and dairy products, plans and methods for judging dairy cattle and the securing and awarding scholarships for students judging contests and of graduate scholarships, and means and methods for the efficient administration of dairy extension work. All these activities have been effective means to bring us closer together in our work, to unify our efforts, to make more effective our results, and to stimulate our enthusiasm."

It may be of interest to our members to know that the influence of our Association is still being felt in the making of laws relative to dairying. The legislative bodies of New York State at their last session carefully studied the well-prepared report of one of our recent committees on the

standardization of milk. A law on this subject has not yet been made in New York. When it is written, however, this report of our Association will have its influence. This is only one illustration of the important position that is held by the American Dairy Science Association in the dairy industry. There are many other examples of the value of our organization to dairying that should be mentioned.

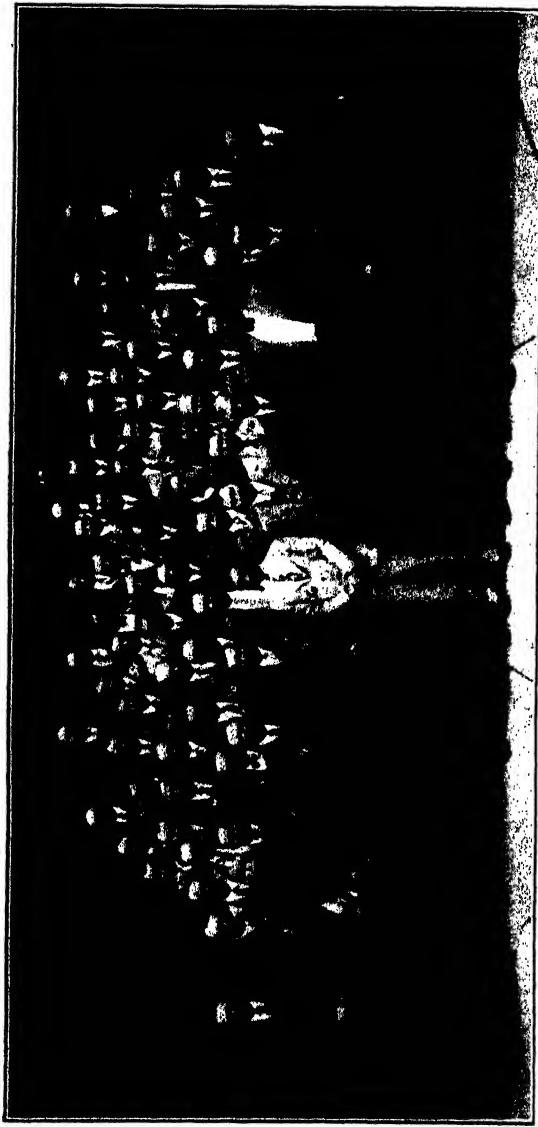
The time came again for a change in the name of our organization. There is a letter under the date of March 19, 1917, that is pasted in the secretary's records, which contains this important final paragraph: "As the result of the action of the Society at its last meeting and as the result of the vote which has just been taken, the 'Official Dairy Instructors' Association' has become the 'American Dairy Science Association.'" This communication was signed by W. A. Stocking, Jr., President, M. Mortensen, Secretary, and H. A. Harding, whom I suppose was the chairman of the committee on the choice of a new name.

The most important development through the years was the establishment of the *JOURNAL OF DAIRY SCIENCE*, the first copy of which came to our desks in May, 1917. I would like to spend several minutes on this significant feature of the life of our Association. I think that it is apparent, however, to all of our members.

Now may I call attention to our meetings and programs. There were a few summer gatherings during the first years of our history. Most of the early meetings, however, were one-day programs that were held some time during the National Dairy Show. It was convenient, of course, for our members to attend the Show and our convention in one trip. Nevertheless, from the standpoint of a good convention, it was not a satisfactory arrangement. The day's work was made up largely of a few papers and discussions and committee reports. Some years the committee reports crowded the papers clear off the slate.

Something had to be done to change this condition of our programs, so we again tried the summer plan in a meeting at Michigan State College in 1927. If I recall correctly, it was a two-day session. This meeting met with the approval of all our members who attended, so we have continued it. In terms of today, our attendance was not large. From the viewpoint of quality you may observe the second picture. One new feature that came to us at our summer conclaves was that of having our families with us. That new departure which adds to the social life of our meetings makes our conventions almost old home-comings.

About this time, in the development of our Association, research, and problems in teaching were rapidly increasing, with the result that many papers were listed on the programs. In fact, valuable discussion has almost been smothered out. Our program committee, however, is endeavoring to return it to its proper place.



AMERICAN DAIRY SCIENCE ASSOCIATION, MICHIGAN STATE COLLEGE, 1927.

- (1) W. H. E. Reid, (2) Otto Schaeffer, (3) W. H. Martin, (4) J. H. Frandsen, (5) George White, (6) J. B. Fitch, (7) J. M. Sherman, (8) C. H. Eckles, (9) H. A. Ruehe, (10) W. M. Regan, (11) O. E. Reed, (12) H. P. Davis, (13) E. L. Anthony, (14) R. B. Stoltz, (15) George Gerbach, (16) , (17) , (18) A. J. Cramer, (19) R. S. Breed, (20) , (21) J. C. Henning, (22) A. C. Baltaser, (23) , (24) , (25) S. I. Bechdel, (26) M. J. Prucha, (27) E. B. Meigs, (28) , (29) , (30) L. H. Addington, (31) H. G. Lindquist, (32) P. S. Lucas, (33) R. J. Harris, (34) A. C. Dahlberg, (35) G. E. Holm, (36) E. J. Perry, (37) W. A. Wentworth, (38) W. M. Neal, (39) , (40) A. C. Ragsdale, (41) P. F. Sharp, (42) P. A. Downs, (43) , (44) , (45) , (46) A. M. Harmon, (47) L. A. Fairchild, (48) R. C. Poits, (49) , (50) H. W. Cave, (51) W. E. Krauss, (52) O. C. Cunningham, (53) W. V. Price, (54) E. S. Guthrie, (55) C. E. Wyile, (56) A. R. Merrill, (57) L. M. Thurston, (58) N. S. Golding, (59) H. O. Henderson, (60) G. M. Trout, (61) , (62) F. W. Bouska, (63) D. S. Kochheimer, (64) A. E. Perkins, (65) J. C. Marquardt, (66) , (67) , (68) J. A. Nelson.

A paragraph should be given to our officers. In the early days we elected the president, vice-president, secretary-treasurer, and editor at the convention instead of using the method of the whole-membership ballot through the mail, as is our practice now. At that time there was no board of directors. In order to obtain better management of our growing organization, the Association decided, after a few years of evolution in administration, to elect a revolving board of six directors. Two new members of this board are elected each year, and two members retire each year after three years of service. The elective administrative members include also the president, the recently retired president, and the vice-president. After a period of service of one year the vice-president automatically becomes our president. The secretary-treasurer is appointed by the entire board of administration and he has an important place in the transaction of the business of the Association. The editor also is appointed now by the whole board of administration on the nomination of the journal Management committee.

Greater continuity of management has been acquired by this new type of administration. The dispatch of business operation has been greatly increased. Particularly satisfactory has been the procedure of passing all committee reports through the hands of our secretary.

The main channels for individual service are through committees. Our Association has been blessed with many members who have been willing to give time and thought to the activity of our organization. They have found many opportunities, for the charter members left the first convention with seven standing committees. We assemble at the thirty-fifth annual convention with 59 committees and 218 committeemen. Fifty of our members are serving on two committees. Seventeen of them have been appointed on three committees. Six of our representatives are laboring on four committees. Three industrious scientists are floundering along on five committees. P. A. Downs is in a class by himself for he is on six committees. Our Association is grateful to these loyal men.

One of the most successful efforts of our journal has been that of the abstracts of literature. The abstractors number 104. They review articles in a concise fashion that are selected from 68 journals and 9 special publications. Among these generous persons there are 65 who are serving on one or more of our committees. It is needless to say that we owe them all a debt of gratitude.

I am sure that our board of directors will join me in paying special recognition to the zeal and wisdom of the officers of the Dairy Production, Dairy Manufactures, and Extension Sections. I, also, know that our board will voice with me a parental solicitation of "good wishes" to the Southern, Eastern, and Western Divisions of our Association.

I wish that I, with 34 years of interest and activity in this Association, could successfully transmit a special message to our younger members. Per-

haps, at this stage of my address, I can gracefully refer to the call of our second president for group study.

During the last four years it has been my privilege to be one of a three-man team on a certain research problem. We have met with greater success than if we, each, had worked alone. This has meant to me a special effort throughout the period to accommodate my plans to those of the majority in order to maintain good teamwork. I, of course, have realized that the other men were doing likewise. Generally, I think that a member of a team should expect to meet the convenience of the others, at least 75 per cent of the time. It is very necessary that he should go more than half way in time, ideas, and the like that pertain to the work of the team. For illustration, a week ago yesterday I found shortly after eight o'clock in the morning that the team wanted to run two sets of milk in our study of deaeration. As it happened, that was commencement day and I had hoped to remain at my desk for a while and then to go and pay honor to the graduating class. Instead, I immediately jumped into my white suit. I ate my lunch as I worked, and I left the laboratory at 6:15. I did not feel badly because of the change in my plans. Naturally changes come. I am accustomed to them. The recompense is greater accomplishment.

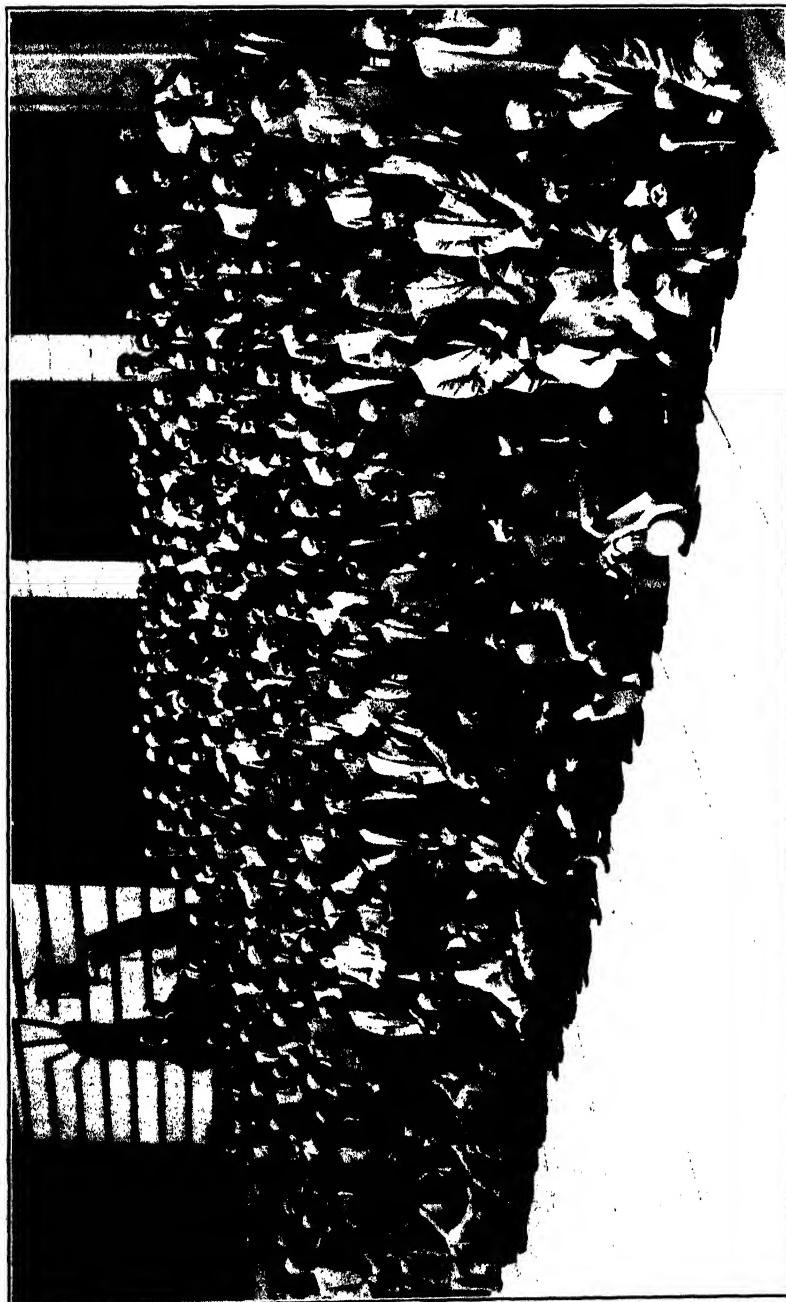
One of the fundamental factors in group activity is the disposition of the teammates. I find it ever present and radiating. It bothers me. Not long ago I heard a question that was being asked in my native state. "Why does Iowa have more Scotchmen than Missouri, and why does Missouri have more mules than Iowa?" The answer is: "Missouri had first choice." If in training my mind and in gaining intelligence, I only had made a wee bit of an effort to enjoy a good joke, to tell satisfactorily a humorous story, or to catch a spark of humor in a stern situation; if only in some way I had been led not to see first the dark side of a new proposition, but rather to recognize immediately the brighter aspects; perhaps then my contributions to the group effort would be appreciated. It might be that I would be less aggravating to my teammates. Possibly then I could help iron out a few of the little difficulties that occasionally occur as the days follow each other.

The endeavor of our Association for united effort in dairy science has extended in personal interest from the 17 charter members of the first picture to over 1500 members, many of whom may be found in the third picture which will be taken during the convention here at Purdue University.

There were 265 members present. The meeting adjourned at 11:30.

GENERAL MEETING OF THE  
AMERICAN DAIRY SCIENCE ASSOCIATION  
*West LaFayette, Indiana, June 27, 1940*

President Guthrie called the meeting to order at 4:00 P.M. in the Purdue Memorial Union Building, there being 204 present, and gave the following address:



We are assembled now to pay homage to the memory of Dr. S. M. Babcock, in this program commemorating the "50th Anniversary of the Babcock Test." Naturally when we realize the value of certain contributions of science to the dairy industry we think of the efforts of many individuals.

A few years ago I heard Dr. J. L. Hills, Dean of the College of Agriculture of Vermont, say that as a young chemist he was well on the way of developing a test for milkfat in dairy products when Babcock's method appeared. The demand of the times had stimulated these two men to work on that problem simultaneously. These studies occurred in the lifetime of a man who is still active, so we must know that we are employed in a rather youthful field.

Dean Hills related how he went to a dairy plant one morning to obtain samples of milk where there were forty-one patrons. Forty of these milks had been skimmed. He talked to the people about the practice and found that one reason for the milk being particularly thin that day was because there was to be a church strawberry social that night.

I am planning, during our convention next year, to visit some of the cemeteries in New England and commune with some of my ancestors on some of their religious standards.

Dr. Babcock was our guest of honor at the banquet when our convention was held at the University of Wisconsin in 1928. We all returned to our homes with pleasant memories—memories of Babcock the scientist, and Babcock the man.

It is now my privilege to present to you Dr. H. C. Jackson, chief of the Department of Dairy Industry, who will have immediate charge of the program.

Dr. Jackson then responded:

Fifty years ago this June, Dr. S. M. Babcock gave to the dairy world the test which bears his name. To many of us who had the honor and pleasure of associating with Dr. Babcock it seemed fitting on this 50th anniversary to devote a portion of the program of the association in commemoration. In these troubled times when whole nations appear to be questioning the very fundamentals underlying our civilization it is refreshing to review the life of a great scientist who pioneered in the field of Dairy Science and who used his gifts for the improvement of not only the lot of dairymen but humanity as well. It is hoped that such a review of his life and activities will serve as an inspiration to the younger members of the association who are just entering their life's work as dairy scientists and that some who are older may have their courage and faith renewed.

Dr. Babcock was a great scientist, a great teacher, and a great man. We are fortunate indeed in having as a speaker on this program a man, a mem-

ber of the association, who was intimately associated with Dr. Babcock in his work at Wisconsin. I know of no one who is better able to pay a tribute to Dr. Babcock than Professor E. B. Hart, Chairman of the Department of Agricultural Chemistry of the University of Wisconsin, who will address us on "Dr. Babcock, the Scientist."

Professor Hart then said :

If Dr. Babcock were with us today he would wish that we have the merriest time possible. For as Glenn Frank characterized him, "He was the Laughing Saint of Science."

Dr. Babcock spent more than forty years in our midst and it was a great experience to know him well. I first met him in 1906, the year in which I was asked to become associated with him in discharging the duties of the Department of Agricultural Chemistry. I shall never forget his trudging the streets with me looking for a house in which to live and taking as much personal interest in me as a father. I learned to know that that was his nature—a helpful spirit always giving sound counsel and always willing to lay aside his own work to discuss your problem. With my coming to the department he was freer to work on problems of no immediate practical moment, and we found him active in his laboratory on the fundamental questions in plant and animal physiology, namely, metabolic water. This piece of work is now considered a classic by plant physiologists, and to animal physiologists it explains how a clothes moth can live on dry clothes and produce larvae containing 75 per cent of water. Dr. Babcock's grip on science was thorough, for he had had superb training at Cornell University and the University of Göttingen, Germany, after having taken his A.B. degree at Tufts College. He brought to his problems a thorough understanding of mathematics, physics, and chemistry.

He always delighted in telling his friends of his experiences in Göttingen University where he was a student of Huebner, the successor to the great chemist Wöhler. Wöhler was then a very old man, revered by the students who knew him, and almost daily walked through the laboratory where Dr. Babcock was at work. On one occasion Dr. Babcock asked a fellow German student, "Who is that old duffer shuffling through here?" To be replied to in scorn, "Don't you know that that is the famous Wöhler?" In Wöhler, Dr. Babcock found a most lovable man, and he never tired of telling how Wöhler showed him a sample of the first urea that was synthesized and a bit of iodine that was sent by Courtois in Paris to Wöhler. It was a sample of the first iodine that had been isolated and it was in a small sealed glass tube. The sample was so minute that it appeared as a small speck in the tube and only by warming the latter and causing the violet vapors to form could one be sure that the tube contained iodine.

Dr. Babcock also visited Bunsen at Heidelberg and was greatly impressed

by the simplicity and originality of that great chemist. I have always suspected that much of the simplicity of his own experimental work grew out of the impressions he received from those early chemists.

In the popular mind Dr. Babcock will be remembered longest for his invention of the milk fat test which bears his name. He has told me many times that his real contribution to the development of this test lay in his introducing the centrifuge as a part of the test, thereby shortening the time of operation. Here again his fundamental knowledge of physics stood him in good stead. Probably no contribution to agriculture by a scientific man has helped more to gain the respect and confidence of farmers in agricultural experiment stations than the Babcock test. But this inventive genius also developed a viscosimeter, the construction of which in principle is the basis of the modern viscosimeter. He chuckled and laughed as he told of this invention, for after it had been perfected to his satisfaction he found that the same principle and the same type of instrument had appeared in the German literature some twenty years before his own invention.

Dr. Babcock was a thoroughly religious man—if we may define a religious man as one who is humble before the Unknown, rather than a blind follower of theological ritual. In his early life he lost a job because he would not take part in the religious exercises prescribed for students at a certain eastern institution. He had been recommended to the position of chemist at this institution and while looking over the position was entertained at the president's house and was definitely offered the job. On the morning that he was to depart from the institution, and was having breakfast with the president, the president said, "Now, Mr. Babcock, there is one thing that I haven't mentioned to you and that is that all members of our faculty take part in the daily chapel exercises held for our students, and of course you will be expected to take your part." Dr. Babcock, with a merry twinkle in his eye, said, "Mr. President, if I come to this institution I come as a chemist and not to take part in these religious exercises," and with that understanding he went back to Cornell University never expecting again to hear from the president of the eastern institution. In a few days he did hear that he had been appointed to the position, but he said that he was very glad that he could inform the president that he had already accepted a position at the Geneva Experiment Station.

Dr. Babcock was a thoroughly practical man although sometimes his advice bordered on the ridiculous. I well recall the incident of one of our faculty leaving for the Pacific Coast to lay out a new city below San Diego. Doctor was sought for advice on what he thought might make a model city. He believed that it would be impossible in that climate to have sufficient milk for a growing population as produced under ordinary circumstances and so implored his friend to try the novel experiment of domesticating South Pacific whales to give up their milk to be pumped through a pipeline from

the ocean slip. So far as I know the experiment was never carried out, but it illustrates the originality of the Doctor and his willingness to try what he often called "the fool experiment." He symbolized what A. V. Hill once said, "It is dangerous to speculate too far, but it is foolish not to speculate at all."

In Dr. Babcock's life there was no lull in activity and interest in scientific problems. He never seemed to grow old. At 87 he was still active on the problem of the constitution of matter, a problem which had always interested him since his student days at Cornell. It has always seemed to me that the many things he talked of in reference to the constitution of matter 30 and 40 years ago when I first knew him have come to be a part of modern physics and modern chemistry. True, he was crude in his experimentation, but the ideas were there waiting for someone to prove or disprove. Physicists often quote the famous Morley experiment refined and carried out to the fifth decimal place as giving the atomic weight of oxygen. In some early experiments in weighing gases that Dr. Babcock himself carried out, he saw how a considerable error could creep in through the outside pressure upon the weighing flask, thereby distorting its shape. He wrote Morley asking if he had given consideration to this possible error in his own work, but Morley never replied, being conscious, I think, that he himself had actually committed a serious error. Dr. Babcock only chuckled and went on his way.

When he died he left a manuscript which we still have in the vault and which was the fruit of his labor and thought on the constitution of matter. I have read it through several times, and to me it was a new physics, but after submitting it to mathematicians and physicists upon our own campus with the thought of its publication, they were unanimous in believing that it was too qualitative in its character to warrant its publication and that possibly it might detract from Dr. Babcock's standing as a scientist.

After hearing their verdict I went to each one personally, not knowing enough about the subject myself, and said, "Isn't it possible that there is some hidden gem of thought in this manuscript that has escaped your critical attention and which might be lost if the manuscript were not published?" They all agreed that they did not think that to be the case. I still wonder whether they are right and whether our leading scientists may not often make the error of being grooved in certain channels of thought until they are roused from them by some wholly new and original idea.

His was the inquiring mind under all circumstances. When the tomatoes failed to grow in his garden the cause must be known. Instead of planting in a row, the next crop was planted in a circle and only those plants in the old row died. That row was underlain by an ant run and the cause of failure solved.

Dr. Babcock received many honors. But I don't know of one from which

he had more fun than that which grew out of the Capper award. After it became known to the public that he was to receive \$5,000 plus a medal, he was hounded by many people, including reporters, as to what he expected to do with the money. In his very characteristic way he told them he was going to buy peanuts with it, and of course this story got into the press. Out of it from all over the country came many appeals for financial help. It was evident that many believed that his mind had failed and that he could not possibly be responsible for the further handling of his own affairs. There wasn't a day that he didn't come to the laboratory with a letter or two to tell of the most recent request for help. One farmer in the north had suffered a great fire, his barns were all down, but he had money enough to rebuild everything but the hen coop—and if he could only have \$500 he could rebuild the plant and again be on his way. A daughter was burdened with the care of an invalid mother; they were poor and needed help. The mother needed the out-door air, and if they could only have money enough for an automobile, health would be immediately restored. The Doctor would again laugh—and when he laughed, he laughed all over.

The present century has seen a great development in our knowledge of animal nutrition. Dr. Babcock's contribution to that development is not generally known. He lighted the torch for others to carry on. When chemist at the Geneva Experiment Station in 1882-1888, Dr. Sturtevant, the director of the station, wanted him to make some of the conventional analyses then and still in vogue on foodstuffs. The work involved not only the analysis of the food but also the analysis of some of the metabolic by-products. After he made the analyses and reduced the results to an ash free basis, the composition of the food was much like that of the metabolic by-product. From that time on he lost faith in the possibility of the prevailing methods of food analysis to give valuable information about the nutritive value of a foodstuff. He also had little faith in the then prevailing and developing notion that the energy of a foodstuff would measure its nutritive value. The mind of the skeptic was again at work. He delighted in telling the American nutrition leaders of forty years ago—Atwater, Armsby, or Jordan, all champions of the idea that the energy of a food measured its nutritive value—that if energy were the measure, then hot water or coal should be the most excellent of foods. When he came to Wisconsin he put his ideas to work in testing with cows, rations alike according to conventional methods of food analysis and energy content but selected from different sources, with marked differences in the resultant milk production and behavior of the animals. His notes were incomplete, but from no fault of his own, and so he never published the data. That work was really the forerunner of the larger development at Wisconsin of the newer knowledge of nutrition, and the first experiment with large animals, so far as I know, using the biological method for testing the nutritive value of

foodstuffs. It was a new idea and Dr. Babcock was father of the idea. Others have carried it on. Had the idea been lacking, we might still be in the hands of the energy exponents.

Dr. Babcock was a most lovable character and exemplified modesty and simplicity in every thing he did. He had a profound influence upon the character of the scientific work done in our college, and possibly the whole University. New ideas, free discussion, kindly interest, and above all his novel and helpful suggestions were an inspiration to many. He never threw a blanket over his apparatus when visitors came to see him. If interested, he hold them fully what he was doing. When he suggested "the fool experiment" he started a new train of thought and out of it came a new birth. He would rather have an experiment fail than succeed if through its failure it taught him to plan for the next. Truth must always prevail. Babcock was not a victim of the routine. The clock could not be regulated by his daily activities. Often he would come to the laboratory to tell me with much enthusiasm that he now had the solution of his problem—and then six months might elapse without reference to it. We thought he had forgotten about it, but not so. The incubation period was on; the first explanation was wrong; he was on a new tack, and soon he would be bubbling over with new enthusiasm. His researches ran in cycles but never ceased.

He represented the liberal in research, and there can be little fundamental research if executive regulations dominate the time of men. His profound understanding and sympathetic nature made him a great teacher, and like Agassiz he is remembered by many whose questions were not answered but who were started on the way to learn for themselves through their own efforts.

Dr. Babcock published little. His influence was felt more through his interests in and suggestions to others. His counsel was always available and his ideas fresh and original. Fourscore years and more did not abate his interest in the experimental work of science. Babcock would have made any institution great, and it is Wisconsin's fortune to claim him for her fame.

Dr. Jackson then introduced Dr. Bohstedt saying:

In the address, which has just been concluded by Professor Hart and his presentation of Dr. Babcock, the scientist, he has given us a brief glimpse of Dr. Babcock, the man. I well recall my first meeting with Dr. Babcock. In the fall of 1927, I was called to the University of Wisconsin to assume the duties of chairman of the Department of Dairy Industry. Although Dr. Babcock at that time was well along in his eighties, he was one of the first to call on me in my office. I remember his saying, "Jackson, if there is anything that I can do for you, don't hesitate to call on me." You can well imagine the impression that this made upon me to have a great scientist

come and offer his services, and the fine thing about it was that he meant it, and that he was in a position to make such a genuine offer. Dr. Gustav Bohstedt, our next speaker, was intimately acquainted with Dr. Babcock. As Professor Hart has pointed out, Dr. Babcock was accompanied by Professor Bohstedt on picnics, various athletic contests, and other social gatherings, and he is certainly well qualified to give us our next address on Dr. Babcock, the man.

Dr. Bohstedt then replied on "Babcock, the Man."

Near the center of the dining room of the University Club in Madison is a table which, by members who knew him, will always be referred to as the Babcock table. Each noonday during the last years of his life, or ever since the death of Mrs. Babcock, the doctor would enter the Club, put his cap in his coat pocket so that later he would not have to hunt for it in any dimly lighted cloakroom, and join a half dozen colleagues at that table.

A vacant chair here seldom went begging. It was a privilege to sit in with a group of men whose conversation sparkled, in large part because it was stimulated by that octogenarian with the infectious laugh who, by common consent, was wont to sit at the end of that table.

Perhaps it was a story on himself, on the other fellow. No matter. It was bound to be entertaining and somehow memorable. More than likely a question in natural history was being debated. Dr. Babcock to the very last was possessed of an insatiable curiosity. "Each morning he met the Universe with a question." What might cause the peculiar pattern of the grain in birdseye maple? What had caused the disappearance of the passenger pigeon? How was that squirrel in his backyard able to find the exact spot where, on digging, it would find a nut buried the previous fall? Did this squirrel reach it through a peculiarly keen sense of smell, or sight, or memory, or sense of location? Various college faculties usually were represented around that table, and each might claim credit for certain of the phenomena under discussion. Dr. Babcock again and again would bubble over with laughter.

With perhaps only two or three left at the table, and things quieting down, there might be some reminiscence of the doctor from his early life. This might have a most serious side, but almost invariably it would yield its quota of mirth.

Last week at that selfsame table, after war and politics had been discussed, and somehow the name of Babcock had been mentioned, was it any wonder that the group present indulged in nostalgic memories of that grand old man?

How could he help but be revered when he was so approachable and friendly? His international fame sat lightly upon this genial and jolly man. Never need a lowly freshman hesitate to stop him on the street for

a talk. Many a budding journalist would interview him, although the doctor as a prank might turn tables on the interviewer, as he did with a personable young lady who later became prom queen. Dr. Babcock found out all about her family and her history, but gave her nothing in return. Too late, the young lady realized the trick that had been played on her. But the experience appealed to her sense of humor and at various times in subsequent years, to the delight of Dr. Babcock, the young lady would call at his home.

Dr. Babcock exemplified that rare class of people who grow old gracefully. He held old friends and continued to make new ones. A certain boyish quality endeared him to young and old. To the very last he was a lover of sports. The autobiography of John L. Sullivan intrigued him. When his eyesight became too dim to follow football and baseball, he would be found in one of the front rows at basketball games. He would discuss the games in terms of the individual players, showing that it was always the human element that interested him, and which explained in part the hold he had on people. He esteemed his fellow man. Personally, I do not recall that at any time during the several years I had frequent opportunities for visitation, Dr. Babcock ever dwelt on meanness in any individual. Although few were better judges of men than he was, such undesirable traits were passed over briefly. I recall one University administrator telling about difficult sessions with faculty members, when his patience might be sorely tried, only to have his faith in mankind renewed the minute Dr. Babcock, himself the epitome of selflessness and good humor, dropped in for a chat. He would laugh away those difficulties.

But to come back to some of the reminiscences of Dr. Babcock, which stories and anecdotes perhaps bring out the man, or boy, as well as anything else might do. Dr. Babcock grew up on a farm in northern New York. The baseball-minded boys were playing catch one noon when somehow the ball rolled under the porch, the base of which had weeks previously been enclosed with some wooden lattice work. Dr. Babcock laughed when he said that, of course, to recover that ball they would have torn down the whole porch. However, the removal of a side panel sufficed. But what did they find underneath the porch beside that ball? A hen! Ever since the panels had been put in place, after weeks of starvation and therefore quite emaciated, the hen was still alive. There arose the question as to how much water she had had to drink during the several weeks. The young farm boy never forgot this instance. In later years this recollection had much to do with his deliberations and researches on the role of metabolic water in vital phenomena, both plant and animal.

The resourcefulness of young Babcock was demonstrated on an occasion when a barn was to be built and for which a tree-trunk at the top of a hill was to be used for a sill. With his typical spirit of independence, he chal-

lenged his family that with the help of a team of oxen, a log chain, and a two-wheeled running gear, he would by himself bring that log down the hill. In telling of this stunt, the doctor explained that when he got to the log, he took one wheel off the axle and put the tip of this axle underneath the heavier end of the log. He thereupon, by means of a log chain and the use of the oxen, rolled the tree trunk on to the running gear, kept those sturdy old oxen pulling until the free axle was lifted, put back the wheel, and delivered the trunk to the building site.

With his tremendous interest in things about him—in people, in the outdoors, in machines and gadgets—was it any wonder that when a student at Tufts College, where he was obliged to study primarily the classics and literature, he felt himself out of his element? In natural resentment he expressed himself in ways that got him into difficulties. Printed rules governing conduct of students would be found torn off the bulletin boards. But on being questioned regarding this, he would truthfully admit this bit of vandalism. His truthfulness more than once prompted leniency on the part of the authorities. But when along with a fellow student he refused to tell on others for hazing freshmen, he was expelled for three weeks. Considering the natural abilities of Dr. Babcock, it may be surprising to have him admit, as he did, that he was graduated at the foot of the class. Not so surprising, in 1901 Tufts College was pleased to grant him an honorary degree of Doctor of Laws. But Latin, Greek, or the history of the ancients interested him far less than things about him. He wanted to be an engineer and not a literary man. Later at Rensselaer Polytechnic Institute, at Cornell, and at Göttingen University, he had opportunities to satisfy his bent for the physical sciences.

Nevertheless, while living at the outskirts of Ithaca where he owned the old Schuyler farm of twenty acres, and while employed on a part-time basis in the Chemistry Department at Cornell, he joined a literary club. Surely the social life must have been as much of an attraction to him as the nominal purpose of the organization. To hear Dr. Babcock tell about the meetings of the club, the membership did, indeed, mix a great deal of fun with their literary efforts. One of the most exciting episodes that I personally ever heard him tell relates to the attempted "kidnapping" of a recently butchered pig belonging to the family of a member of the club. Circumstantial evidence pointed to Babcock as one of the perpetrators, and one may surmise the suppressed hilarity of the members when at the very next meeting of the club, two phony policemen ceremoniously ushered the unsuspecting Babcock into the circle assembled in mock court. In due course he was fined one-half bushel of peanuts. In far off Göttingen a few years later he heard echoes of this affair, which had been written up in mock seriousness. But it had been taken at face value in Göttingen and no doubt various other places, by people who were astonished that a professor in an American University should stoop so low as to steal a pig!

It is enough to appreciate that this, to some of us perhaps, austere and certainly famous scientist has not always been perfect, but rather has been intensely human. Nevertheless, as one of this man's great admirers has said in his behalf, "It's the little imperfections in our heroes that endear them to us."

In his work at Cornell University he was a special adult student, taking no regular classroom courses. But in his laboratory work he was guided by Professor Caldwell. His rapid progress may be inferred from the fact that at the end of three years, he was asked to take charge of the laboratory and teach a general laboratory course in chemistry. Very likely his experience and associations in this laboratory were as important to him as any in his entire course of training. One may assume that this experience greatly stimulated his independent thinking.

Witness an example of his iconoclastic views regarding gross chemical analyses of feedstuffs as guides to nutritive values. While giving his presidential address before the Association of Official Agricultural Chemists he pointed out to them limitations of such analyses, and as a striking example made the statement that, on the basis of a gross chemical analysis, coal had a relatively high feed value. He elaborated before his listeners that coal surely had both moisture and dry matter. Coal further had a certain amount of nitrogen by the Kjeldahl method, which nitrogen multiplied by 6.25 would be reported as protein. Surely coal had ash or mineral matter in it, in many cases too much of this particular ingredient. Likewise fiber would be found, and ether extract. Then by the usual computation of difference, the remaining part of the dry matter would be reported as nitrogen-free extract.

Such evidences of untrammeled thinking must have stunned a good many of his audience. Small wonder that Babcock was one of the first to observe shortcomings of so-called balanced rations, and should have suggested that animals were in need of nutrient principles other than starch or carbohydrates, fats, proteins and minerals. Surely the present knowledge of vitamins owes a great deal to this man of the "seeing eye and inquiring mind."

Quoting from the Babcock funeral oration of President Glenn Frank:

"He pursued the most painstaking research as if he were playing a game. He brought to his tasks that gaiety of spirit which authentic greatness can afford. His spirit never surrendered that incorrigible playfulness which so often marks men of power. He brought laughter into the laboratory, for there was about him that deceptively careless air which creative spirits have as they go about their business. . . .

"In an age smitten with the passion for publicity, he forgot himself into immortality!

"And in the midst of the sickness of an acquisitive society, his spirit remained unsullied even by legitimate personal considerations!"

"Scholar of a great university!  
Servant of a great state!  
Shy benefactor of mankind everywhere!  
Laughing saint of science!  
Being dead he yet speaks!"

## GENERAL BUSINESS MEETING

## AMERICAN DAIRY SCIENCE ASSOCIATION

*West LaFayette, Indiana, June 28, 1940*

President E. S. Guthrie called the meeting to order at 9:00 A.M. in Purdue Memorial Union Building on the Purdue University campus. Mr. Charles Blackman, Chairman of the Necrology Committee, made the following report of the eight members who passed away during the year:

PROFESSOR GEORGE E. MORTON .....	July 11, 1939
F. L. SCHOENBERGER .....	September 2, 1939
J. R. MACLENNAN .....	Winter, 1939
DR. LOYD M. THURSTON .....	February 29, 1940
W. D. AXTELL, JR. ....	March 28, 1940
R. D. CANAN .....	April 19, 1940
KENNETH J. MATHESON .....	April 24, 1940
FISHER STILLEY .....	May 4, 1940

Upon motion duly seconded the report was accepted to be made a matter of record in the minutes.

Editor Sutton then gave a report which will be found in the minutes of the board of directors.

*Manufacturing Section*

Secretary Coulter of the Manufacturing Section presented the following minutes; upon motion duly seconded they were accepted with the exception of that part referring to the milk score-card which was deleted from the minutes.

The manufacturing section held its regular meetings at the scheduled hours and places. F. H. Herzer, the Section chairman, presided. The papers presented developed a well balanced program which held the interest of the group as evidenced by the discussions when time permitted.

The meetings were conducted with dispatch and promptness and adjourned at scheduled times. All papers were presented as announced except M38, M44, M48, and M51.

Mr. Herzer appointed a nominating committee consisting of: O. F. Garrett, T. B. Harrison, and H. Macy, to nominate a vice-chairman and a secretary for the coming year.

Considerable interest was shown in the reports of the various committees. These reports follow:

*Reports of committees:*

1. Committee on Chemical Methods for the Analysis of Milk and Dairy Products, L. C. THOMSON, *Chairman.* Accepted and written report attached.
2. Committee on Judging of Dairy Products, G. M. TROUT, *Chairman.* Accepted and written report attached.
3. Committee on Methods of Determining the Curd Tension of Milk, F. J. DOAN, *Chairman.* In the absence of any member of the committee this report was not presented; however, the written report was received and is attached.
4. Deleted.
5. Committee on Sanitary Procedure, L. H. BURGWALD, *Chairman.* Accepted and written report attached.
6. Committee on Methods of Measuring the Color of Milk, O. F. GARRETT, *Chairman.* Accepted and written report attached.
7. Committee to Study Methods for Measuring the Oxidation of Milk Fat, O. F. GARRETT, *Chairman.* Accepted and written report attached.
8. Committee on Quality Program, W. H. E. REID, *Chairman.* Accepted and written report attached.
9. Methods for the Bacteriological Analysis of Milk and Dairy Products, H. Macy, *Chairman.* Accepted and written report attached.  
Upon the motion of the committee chairman, the Section voted that the committee on Methods for the Bacteriological Analysis of Milk and Dairy Products be discharged and that a new committee of three, designated "The Committee on Microbiological Methods for the Analysis of Milk and Dairy Products," be appointed and that such committee be empowered to appoint such sub-committees as may be necessary to carry on the work of the committee.
10. Committee to Study Ways for Improving Summer Meetings of Manufacturing Section, B. E. HORRALL, *Chairman.* Accepted and written report attached.

Initiated by M. E. Parker, there was considerable discussion about the type of program which should be presented at the Section meetings. The report of the Committee to Study Ways for Improving Summer Meetings of the Manufacturing Section was called for and presented by B. H. Horrall, Chairman. The Committee made three recommendations:

1. That mimeographed sheets be used for the presentation of data in preference to photographic slides.
2. That speakers be requested to speak extemporaneously rather than to read a prepared manuscript.
3. That the first and last paper on the program at each meeting be of the symposium type.

The sentiment of the Section as expressed by vote was favorable to a program in which a portion of the time be allotted to papers of the symposium type, a portion to the short type paper and a portion to committee reports. The Section voted to appoint a rotating committee to have charge of formulating programs for the summer meetings.

Mr. Herzer instructed the Committee to Study Ways for Improving Summer Meetings of the Manufacturing Section to meet with the Association Board of Directors to consider the desirability of and means to be used in changing the type of program presented by the Manufacturing Section.

After conferring with the Association Board of Directors this committee reported to the Section as follows:

The Board of Directors of the American Dairy Science Association passed a motion last night to recommend to the sections of the Association that:

The program next year consist of the following:

- (1) General papers
- (2) Symposia, and
- (3) Committee reports

The report of the Committee was accepted by vote of the Section.

The nomination committee placed the following names in nomination for officers for the year October 1, 1940, to September 30, 1941:

<i>Vice-Chairman</i> —L. H. BURGWALD	<i>W. H. E. REID</i>
<i>Secretary</i> —H. C. MOORE	<i>E. O. ANDERSON</i>

A vote by ballot was taken, the secretary, O. F. Garrett, T. B. Harrison, and H. Macy acted as tellers. Mr. C. D. Dahl, present Vice-Chairman, automatically becomes Chairman of the Manufacturing Section.

The following officers were elected: *Vice-Chairman*—L. H. BURGWALD, *Secretary*—E. O. Anderson.

Business session adjourned.

(Signed) S. T. COULTER  
*Secretary, Manufacturing Section*

#### *Extension Section*

Mr. Vergeront, Secretary of the Extension Section, presented the following report which upon motion duly seconded was accepted and ordered to be printed in the minutes:

The annual business meeting of the Extension Section was called to order by R. G. Connelly, Chairman, June 26, at 1:30 P.M.; 36 others in attendance from a total of 28 different states.

During the two-day session, selected papers and reports on various phases of the extension program were presented and were discussed by the

members, led by a panel made up of members who had participated in the program.

The Resolution Committee presented the following resolution:

*Resolved*, we wish to express the sincere appreciation of the Extension Section to Purdue University and especially to each and every member of the Dairy Department, who have done so much to successfully prepare for our comfort and enjoyment.

C. R. GEARHART  
E. H. LOVELAND  
JAMES W. LINN

James Linn, Chairman of the Type Classification Committee, presented a progress report which was favorably accepted, but held over to be again presented to the 1941 meeting after further study by the committee.

E. J. Perry, Chairman of the Sire Committee, presented the report for that group. Each section was taken up separately and discussions for the matter of selective registration and the use of standard forms for artificial breeding association were commended.

Upon the motion of C. G. Bradt of New York a new committee on Dairy Cattle was established.

The Health Report of a joint committee of the Production and Extension sections of Feeding was made by Professor E. S. Savage, Chairman, and was unanimously adopted.

The members of this section expressed themselves as being very much pleased with the Joint Session with the Production Session on Artificial Breeding, and with the Exhibit of Ideas and with the Panel Discussions which were a part of every program of the section.

During the business session of the section, J. F. Kendrick, of the Bureau of Dairy Industry, was elected Secretary. The officers who will assume their responsibilities on October 1, 1940, are as follows:

*Chairman*—OTTO J. HILL, Pullman, Washington

*Vice-Chairman and Chairman of the Program Committee*—

GLEN W. VERGERONT, Madison, Wisconsin

*Secretary*—J. F. KENDRICK, Washington, D. C.

Respectfully submitted,

(Signed) GLEN W. VERGERONT

*Secretary, Extension Section*

#### *Production Section*

Mr. H. A. Herman, Secretary of the Production Section, presented the following report and upon motion duly seconded it was accepted and ordered to be printed in the minutes:

The production section held five regularly scheduled sessions. The first

session, Tuesday afternoon, June 25, was a combined meeting of the production and extension sections and consisted of a symposium on artificial insemination of dairy cows. The remaining formal sessions were devoted to papers grouped under sectional headings of: Milk Secretion, Breeding, Disease, Calf Feeding, and Nutrition, Minerals and Vitamins. Dr. A. H. Kuhlman presided at all sessions.

All sessions were well attended and much interest was exhibited in the papers presented. The papers were well prepared and ably presented with slides and mimeographed material used to supplement the presentation. Fifty-six papers were presented, a record number, as all but two of the 58 scheduled papers were presented. The marked program, attached, indicated the member presenting each paper.

The business meeting of the section was held at 8:30 A.M., Thursday, June 27, in Room 340, Purdue Memorial Union Building, with about 100 members in attendance. Dr. A. H. Kuhlman, Chairman, presided. The minutes of the 1939 meeting at Pullman, Washington, were read and approved.

Reports were submitted by the various standing committees and approved. Copies of these reports attached.

Points of particular interest incorporated in the adopted reports and presented to the general business session for approval are:

*Breeds Relation Committee—J. W. BARTLETT, N. J., Chairman*

1. The present specified numbers of milkings (36) to be supervised daily for cows on Advanced Registry Test should not be increased. It is the obligation of the supervising institution to enforce this rule.
2. No changes in the Uniform Herd Test Forms are recommended at this time.
3. The preliminary milking shall be weighed, sampled and tested for butterfat in the usual manner. The preliminary milking shall constitute a regular milking period with respect to the number of milkings supervised daily.
4. Supervisory institutions in the respective states, not in harmony with breed associations rules more lax than the Uniform Rules may well use the Uniform Rules as a basis for conduct of Advanced Registry and Herd Improvement Registry Tests.
5. To revise and bring up to date the Uniform Rules of the Association concerning the conduct of Advanced Registry and Herd Improvement Registry Testing and to have these printed in the JOURNAL OF DAIRY SCIENCE with sufficient extra copies for breed associations and supervision institutions. It is further recommended that the breed associations print the uniform rules in their rule booklets.
6. "If a cow on Advanced Registry or Herd Improvement Registry test

aborts while in milk, and the gestation is 152 days or longer, her current record shall end and a new lactation shall begin, but if the abortion occurs at less than 152 days the test is to be continued to complete the 305 or 365 day test."

7. The matter of compiling a uniform set of age conversion factors is being investigated further through the cooperation of the Bureau of Dairy Industry, United States Department of Agriculture.

8. The problem of working out a plan of Dairy Herd Improvement Association Type classification is continued with the Breeds Relations and Extension Section committees cooperating.

9. "That the American Dairy Cattle Club be permitted to use the Uniform Head Improvement Registry Test Form, but that only the names of the breeds of dairy cattle recognized in the purebred dairy cattle registry associations be printed on the form."

10. Supervisors conducting official tests should conform to the same requirements with respect to health certificates as is required of all milk handlers in accord with the provisions of the Standard Milk Ordinance, United States Public Health Service.

11. That close cooperation be maintained between the Breeds Relations Committee and the respective dairy cattle breed associations with respect to the uniformity of registering offspring resulting from artificial insemination.

12. That all, except current records of the Breeds Relations Committee be bound and filed with the Secretary of the American Dairy Science Association, as a part of the productions sections records, for safe keeping.

In view of the large number of papers presented and the inadequate time for business matters it was recommended and approved that this section go on record as being favorable to preparation of the program for the Production Section, and that the program be arranged, if necessary, in sections running concurrently, and that more time be provided for business meetings.

Committee on program to consist of Production Section Chairman and Secretary, and one member to be appointed by the chairman, but so appointed that the committee is continuous in operation, and only one new member be appointed each year.

All Standing Committees were reappointed with their present personnel except as follows:

Breeds Relations Committee, W. W. Yapp of Illinois appointed for a period of three years to succeed J. W. Bartlett, and H. A. Herman, serving unexpired term for Earl Weaver, appointed for three years, W. T. Crandall, Cornell, appointed chairman.

A. A. Borland, chairman of the Nominating Committee, presented names for offices of Vice-Chairman and Secretary of the Section for 1940. H. A. Herman, Missouri, was elected Vice-Chairman, and K. S. Morrow, New

Hampshire, was elected secretary. W. E. Petersen of Minnesota, Vice-Chairman 1939-40, automatically becomes chairman for 1940-41.

Respectfully submitted,

(Signed) A. H. KUHLMAN, *Chairman*  
H. A. HERMAN, *Secretary*

Mr. Horrall, Chairman of the Program Committee, presented the following report which, upon motion duly seconded, was accepted:

The Program Committee for the 35th Annual Meeting of the American Dairy Science Association consists of F. H. Herzer, Mississippi State College, T. S. Sutton, Ohio State University, E. V. Ellington, State College of Washington, A. H. Kuhlman, Oklahoma A. and M. College, R. G. Connelly, Virginia A. and M. College, and B. E. Horrall, Purdue University, Chairman.

The first call for titles for papers was made January 4, 1940, in a letter addressed to the heads of Dairy and Animal Husbandry Departments, Bureau of Dairy Industry and Secretaries of large Associations. A call for abstracts was made in the January, February, March, and April numbers of the JOURNAL OF DAIRY SCIENCE. All abstracts were due to be in the hands of the Program Chairman on or before April 15, 1940, to be included in the program.

Six abstracts were received after the deadline of April 15. These were immediately sent back to the authors with our regrets that the abstracts were received after the deadline set by the committee.

There were received before and on April 15, 126 abstracts, and they were distributed as follows: Manufacturing, 51; Production, 58; and Extension, 17.

(Signed) B. E. HORRALL  
*Chairman*

#### *Resolution Committee*

Mr. Ragsdale, Chairman of the Resolution Committee, presented the following report, which, upon motion duly seconded, was accepted:

The American Dairy Science Association assembled in its 35th annual meeting at Purdue University wishes to express its appreciation for the hospitality, delightful entertainment, and splendid facilities provided by the officials and faculty of that University.

Therefore, be it Resolved: That the membership of the Association publicly express its sincere appreciation to President E. C. Elliott; H. J. Reed, Dean and Director; Professor H. W. Gregory, and members of the departmental staff; A. P. Stewart, Director of Music, all of Purdue University, and to all agencies and organizations which cooperated with them in providing programs and entertainment, and in extending so many fine courtesies.

WHEREAS, the American Dairy Science Association feels that it is an inspiration to all and especially to the younger members to have the presence of those who have been long associated with this association:

Therefore, be it *Resolved*: That we note with appreciation the great regularity of attendance of W. J. Frazier, C. C. Hayden, O. F. Hunziker, and E. S. Guthrie, charter members; J. F. Frandsen, founder of the JOURNAL OF DAIRY SCIENCE, and those whom the association has seen fit to give honorary recognition because of long and exceptional service and leadership; namely, W. J. Frazier, O. F. Hunziker, M. Mortensen, and J. F. Frandsen.

The American Dairy Science Association also wishes to express its appreciation to the Borden Company for its continued interest in dairy research as indicated by its continuing awards for superior research in dairying and related fields.

The American Dairy Science Association would like finally to express its appreciation to the members of the program committee who have been responsible for the organization of one of the finest programs ever presented. The members of this committee being F. H. Herzer, T. S. Sutton, E. V. Ellington, A. H. Kuhlman, R. G. Connelly, and B. E. Horrall, Chairman.

Submitted by  
G. A. BOWLING  
H. A. HARDING  
C. C. HAYDEN  
C. A. IVERSON  
A. C. RAGSDALE, *Chairman*

Mr. Borland reported for Mr. Brueckner, Chairman of the Committee on the Time of Meeting, and the report will be found in the minutes of the Board of Directors.

Mr. Boxell, Chairman of the Registration Committee, reported the following:

The 1940 figures represent a total of 715 men, women, and children. Of the 480 men, approximately 388 registered as active members. 140 were listed as representatives of commercial concerns. This is only a preliminary report. The above figures are believed to be a trifle high. A complete and verified report will be submitted to the secretary at the earliest possible moment.

(Signed) K. C. BOXELL

The Secretary then gave the summary of his report.

The policy of this association is to have the Board of Directors handle most of the business of this association. However, it is the opinion of the Board that all reports that have been accepted by the Board of Directors should also come before this business session so that the membership will know as early as possible the action that the Board has taken. A copy of

the Certified Public Accountant's Audit was made last January 25 and mailed to each of the Board of Directors. I will not burden you with the details of this report but I should like to compare for you the income of 1935 with 1939. In 1935 it was \$7,873 and last year it was \$17,572. The report of the auditing committee was then read. See meeting of Board of Directors.

This year we are publishing 2800 journals each month instead of 2500. The Board of Directors have recently authorized the expenditure of almost \$900 to reproduce nine different numbers of the JOURNAL, including all six numbers of Volume I. Our circulation in 1939 was 2,438. We had 1465 members; 706 subscribers; 73 associate subscribers and 156 affiliates. This year we have taken in 50 new members. Illinois is leading the list with 10; Ohio, 5; New Jersey, 4; California, Indiana, and Minnesota, 3 each; Mississippi, Montana, Pennsylvania, Utah, Wisconsin, 2 each; and those having one are Alabama, Arizona, District of Columbia, Iowa, Kentucky, Louisiana, Massachusetts, New York, South Dakota, Texas, Vermont, and Quebec.

This year President Guthrie appointed a membership committee and most of the efforts were in the direction of stressing the student affiliates. Last year we had 156, and this year thus far we have a total of 247. Iowa led the states with 47 affiliates, Ohio, 34; Illinois, 19; Washington, 16; Wisconsin, 13; Pennsylvania, 12; South Carolina and Texas, 11; New York and Virginia, 10; Indiana, 8; Massachusetts, 7; Connecticut, 6; Maryland, Michigan, Minnesota, 5; California, Missouri, Nebraska, and Tennessee, 3; Idaho, Oregon, Kentucky, and Vermont, 2; Georgia, Kansas, Montana, New Hampshire, New Jersey, Oklahoma, South Dakota, and West Virginia, 1.

The minutes of the Board of Directors were then read.

Upon motion duly seconded the minutes of the Board of Directors were accepted and the association approved and endorsed all action that the Board had taken during the past year. The Secretary then called their attention to the change in the By-laws made at the business meeting yesterday at 4:00 P.M. The section 3, Article 5 was amended to read as follows:

#### Amendment of Section 3, Article 5.

The present section reads as follows: "Section 3—the Quorum of any meeting of the Association shall consist of not less than ten per cent (10%) of the membership."

We propose to amend this section to read five per cent (5%) in lieu of ten per cent (10%) so that section 3 will read as follows:

"Section 3—the Quorum of any meeting of the Association shall consist of not less than five per cent (5%) of the membership."

Back copies of the Journal are now available. It has been suggested that each investigator see whether or not your library has all the back copies of the Journal. They are now procurable from the secretary's office, price

\$5 per Volume up to 1933, \$6 per Volume since 1934. Single copies may be procured at \$1 each.

The report of the Journal Management Committee was then read. For this report see the minutes of the Board of Directors meeting.

Mr. Ellenberger then gave the following report of the Committee on Dairy Curricula:

Speaking for the general committee, I may report that much work has been done by way of collecting and tabulating for study: (1) The objectives of all colleges offering curricula in agriculture and of departments in which students may take majors in any line of dairying, and (2) the individual courses offered in each curriculum.

The existing variations as regards objectives, conditions, and facilities in the different states have been found to be so great that more detailed study must be made. Such study involves much work and time but progress is being made as rapidly as possible.

Lengthy, well-attended committee sessions have been held here at Purdue resulting in much unanimity of opinion. Before the submission of recommendations to this body as to minimum subject matter requirements and curricula it has been decided that certain sub-committees should be set up to make detailed studies and suggestions as to minimum subject matter (not course) requirements in each of the following fields:

For both Production and Manufacturing:

- Chemistry
- Mathematics and Physics
- Bacteriology
- English and Public Speaking
- Economics and Business

Recommended Electives

For Production:

- Biological science including Genetics
- Veterinary subjects as Anatomy, Physiology, Hygiene, and Pathology
- Dairy Production subjects including Nutrition and Feeding, Agricultural Engineering and Mechanics

For Manufacturing:

- Dairy products manufacturing subjects
- Dairy mechanics
- Subjects providing an agricultural background

Committees are to be appointed promptly to consider all these subjects and report their findings by January 1, 1941. After existing committees have considered such reports in connection with other studies they are making, it is expected that an extended mimeographed statement, including rec-

ommendations will be submitted to all members previous to or at our next annual meeting.

The committee has taken cognizance to the facts that (1) there are three divisions of work in our land grant colleges, teaching, research, and extension, (2) the latter two are well provided for in the programs for our annual meetings and (3) this group, though originally organized as the Official Dairy Instructors Association now gives scant consideration in its programs to problems associated with teaching. After conferring with numerous association members and finding seeming unanimous approval this committee hereby requests that the president as chairman of the program committee appoint a committee of three to (1) prepare and submit a program on instruction for at least a one-half day session at our next annual meeting, to run concurrently with programs of the now established sections, and (2) bring before those who may attend that session a proposal for the establishment of a teaching or instruction section, said committee members to act as officers of the unorganized group for the coming year.

Respectfully submitted,

(Signed) H. B. ELLENBERGER

*Chairman*

Mr. C. E. Wylie then gave his report on the Curriculum on Production:

The committee has continued its study of dairy production curricula according to the progress report made at the American Dairy Science Association meeting at Washington State College, June 30, 1939.

1. The committee has initiated a special study of elementary dairy instruction which has been referred to the General Curriculum Committee and is being handled by C. E. Wylie and H. P. Davis.

2. The committee has divided its work and made assignments as follows: Objectives in teaching, C. Y. Cannon—Fundamental courses, I. R. Jones—Required dairy and agricultural courses, J. W. Bartlett—Elective courses, Chas. N. Shepardson—Relation of dairy manufacturing courses to dairy production courses, T. E. Woodward.

3. The committee recommends that several papers be presented on the following subjects at the next annual meeting of the association, and that not less than one-half day be devoted to dairy instruction.

- a. Methods and materials in teaching dairy husbandry—3 papers.
- b. Curriculum construction.
- c. Testing and Revising a Curriculum.

Committee: J. W. BARTLETT, I. R. JONES, C. N. SHEPARDSON,

T. E. WOODWARD, C. Y. CANNON, W. L. CLEVENGER, and

C. E. WYLIE, *Chairman*.

Mr. Mortensen submitted the report signed by Mr. Roadhouse on the Curriculum on Manufacturing.

In continuing the work of the subcommittee on Dairy Manufacturers Curriculum during the present year, a copy of the last annual report was sent to all Dairy Departments with a request for comments. The report emphasized that an effort should be made to develop greater uniformity of dairy manufactures curricula which include sound basic training in order to give a good general education. The report also covered two points of view concerning the curriculum in Dairy Manufactures.

1. That there should be a special curriculum for students majoring in Dairy Manufactures.

2. That there should be one curriculum that would serve both manufacturing and production field in basic training and allow a liberal choice of electives to serve the needs of students specializing in the separate fields.

Dairy Departments of 24 states have responded. After analyzing these replies it appears that in those states where dairy manufacturing is less specialized a combined curriculum for both dairy production and dairy manufacturing is favored; in other colleges a separate curriculum for dairy manufacturing is preferred. The replies indicate that the instruction offered at the different colleges in applied subjects is adequate for the conditions that exist in the individual states. The greatest difficulty occurs when students wish to transfer their credits to other institutions. These students often find themselves handicapped by not having the necessary prerequisites for upper division and graduate instruction.

It is recognized that the demands of the dairy industry in the different states make difficult the adoption of a uniform curriculum. This applies to the policies of the universities and colleges of the different states. With these conditions in mind, it seems desirable that this study be continued with a view to setting up two curricula, one for institutions where dairy production and dairy manufacturing are combined in one department and serve both phases of dairying and a second in which a separate curriculum is recommended for those colleges where dairy manufacturing is taught in a separate department.

In spite of the difficulty of setting up a curriculum uniform in all particulars it is recommended that curricula be made uniform with respect to minimum basic requirements. The committee is proceeding to develop recommendations for outlines of minimum requirements in basic courses and whether there should be different requirements for Manufacturing than for Production.

(Signed) C. L. ROADHOUSE, *Chairman*

The Nominating Committee made the following report:

For Vice-President: H. F. JUDKINS, New York; J. A. NELSON, Montana

For Directors: H. B. ELLENBERGER, Vermont; C. E. WYLIE, Tennessee;

KENNETH N. RENNER, Texas Tech.; A. C. DAHLBERG, Geneva

Committee:

J. H. FRANDSEN

(H. G. Lindquist, acting  
for Frandsen)

T. B. HARRISON

RANDALL WHITAKER

J. A. NEWLANDER

H. W. GREGORY

The Nominating Committee stated that inasmuch as the Association now had 1,465 members made up of approximately 125 men from institutions connected with dairy manufacturing and 140 men from institutions connected with dairy production and 820 men from industries, the committee wishes to submit the name of a man connected with the industries and who was formerly connected with an educational institution.

*Honorary Member*

At the banquet on Thursday night, President Guthrie introduced Mr. Ellenberger who said:

In years past this association has paid honorary tribute to three of its members who, with their wives as partners, and not silent partners either, have rendered outstanding service to our association and to the dairy industry. I am requesting them to rise so that all, particularly the younger persons, may know them by sight as well as by reputation.

Professor William J. Fraser of Illinois, the founder of our organization, was presented a framed "token of appreciation" in 1933 for his "clear vision of the possibilities in advancing the cause of scientific dairying through a closer organization of the workers in the field" and for eminent service to the industry.

Dr. Otto F. Hunziker of Chicago was presented a similar "tribute" in 1934 for "outstanding leadership in dairy research and education" and in association affairs.

Dr. Martin Mortensen of Iowa was given, in 1935, a framed "tribute" in recognition of his "eminent service to the dairy industry . . . and his devotion to our association."

Now, five years later, in 1940, and in accordance with the decision of the awards board comprised of the three most recent past presidents, we are to honor and present a tribute to another of our members, Professor Julius H. Frandsen of Massachusetts.

Professor Frandsen was born in Story County, Iowa, the central county of the central state of the cornbelt. He was raised on a farm and has been associated with dairying in one form or another all of his life. He is a graduate of Iowa State College in the class of 1902, where I was a student with him and from which he received a Master of Science degree in 1904. In 1906 Matilda Madison, another Iowa State College graduate, became his wife and a very active life partner.

From 1904-1907 Professor Frandsen was engaged in commercial work for Professor Mortensen. In 1907 he was appointed the first Professor of Dairy Industry at the University of Idaho, where he remained as head of the department until 1911 when he became Professor of Dairy Husbandry at the University of Nebraska. While there he was instrumental in securing the erection of the dairy building, dedicated in 1917, then recognized as the finest building for college dairying in the country and still ranking as one of the best.

Leaving the University of Nebraska in 1921 to become dairy editor of farm papers, he again returned to teaching and research in 1926 as Professor of Dairy Industry at the Massachusetts State College, the position which he now holds.

Professor Frandsen served as president of the Official Dairy Instructor's Association, as this organization was then named, during 1913 and 1914. In his presidential address of 1913 he made a strong plea for the establishment of a Journal to be published by our association in the interest of dairy science and research. From that time through 1914-1916, he at every opportunity advocated the establishment of such a Journal. This ambition was realized, when at its 1916 annual meeting, this association approved the establishment of the JOURNAL OF DAIRY SCIENCE and designated Professor Frandsen as Editor-in-Chief. It is interesting to note that this action was taken in Flint Laboratory, Amherst, Massachusetts, the building in which Professor Frandsen now has his office.

As editor of the JOURNAL OF DAIRY SCIENCE from May, 1917, to January, 1928, Professor Frandsen has been the means of arousing, encouraging, and advancing dairy research in a way and to an extent now recognized as outstanding and important.

We are sorry that, because of illness, Professor Frandsen cannot be here tonight, but I am pleased to read the framed tribute which our president, Dr. E. S. Guthrie, will forward to him.

#### JULIUS HERMAN FRANDSEN

DAIRYMAN

TEACHER, INVESTIGATOR, AND EDITOR

In recognition of outstanding service to dairy science in America, particularly in the conception, advocacy and establishment of the JOURNAL OF

DAIRY SCIENCE, which he so carefully nurtured and successfully edited and managed for eleven years, a substantial and far-reaching contribution of inestimable value to the advancement of dairy research, teaching, and practice, the American Dairy Science Association at its thirty-fifth Annual Meeting at LaFayette, Indiana, this twenty-seventh day of June, one thousand nine hundred and forty, honors Professor Julius Herman Frandsen and presents this tribute.

R. B. STOLTZ,  
*Secretary*

E. S. GUTHRIE,  
*President*

#### MEETING OF BOARD OF DIRECTORS AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ

*Secretary-Treasurer*

8:00 P.M., June 24, 1940  
West Lafayette, Indiana

A meeting of the Board of Directors of the American Dairy Science Association was held in the Memorial Union Building, Monday, June 24, 1940, at 8:00 P.M.

Present: President E. S. Guthrie; Vice-President H. W. Cave; Secretary-Treasurer R. B. Stoltz; Directors, Harold Macy, J. W. Linn, E. V. Ellington, M. E. Parker, C. N. Shepardson, Fordyce Ely.

Absent: Director, Earl Weaver.

President Guthrie called the meeting to order.

Mr. O. F. Hunziker, Chairman of the Journal Management Committee, submitted the following report:

Your Committee on Journal Management beg to respectfully submit the following brief report:

*Financial:* The income and outgo related to the business operation of the JOURNAL OF DAIRY SCIENCE are given in detail in the Auditor's report for the year 1939, which shows a most gratifying increase in the net revenues of the JOURNAL, and copy of which was mailed to every member of the Board by the Secretary-Treasurer.

*Twenty-Year Index of Journal:* At last year's Annual Meeting your Committee recommended the preparation of an index covering the first 20 volumes of the JOURNAL OF DAIRY SCIENCE; we likewise suggested tentative arrangements and terms dealing with the actual work of indexing.

Your Board approved the general idea of the desirability of such an index and referred the matter back to the Committee and editor for further investigation as to definite arrangements to get the work done, with instructions to report our findings back to the Board. This was done. The Com-

mittee was fortunate in securing the services of Dr. H. Macy to take charge of the index work under the terms stipulated in our original recommendation. The Board approved our proposed arrangement and the job of indexing was turned over to Dr. Macy. The author index has been completed and the subject index is in the process of preparation.

*Reproduction of Back Journals:* In order to take care of orders for back numbers of the JOURNAL, the Journal Management Committee recommended to President Guthrie that a sufficient sum be set aside for the reproduction of such back numbers of the JOURNAL as are out of print. There are nine numbers of the JOURNAL that are either entirely exhausted or of which only a few copies are left. Reproduction of these nine numbers will involve an approximate cost of \$870.00. This recommendation has been approved by the Board, authorizing the Secretary-Treasurer to utilize the necessary amount (not in excess of \$1,000.00) for this purpose.

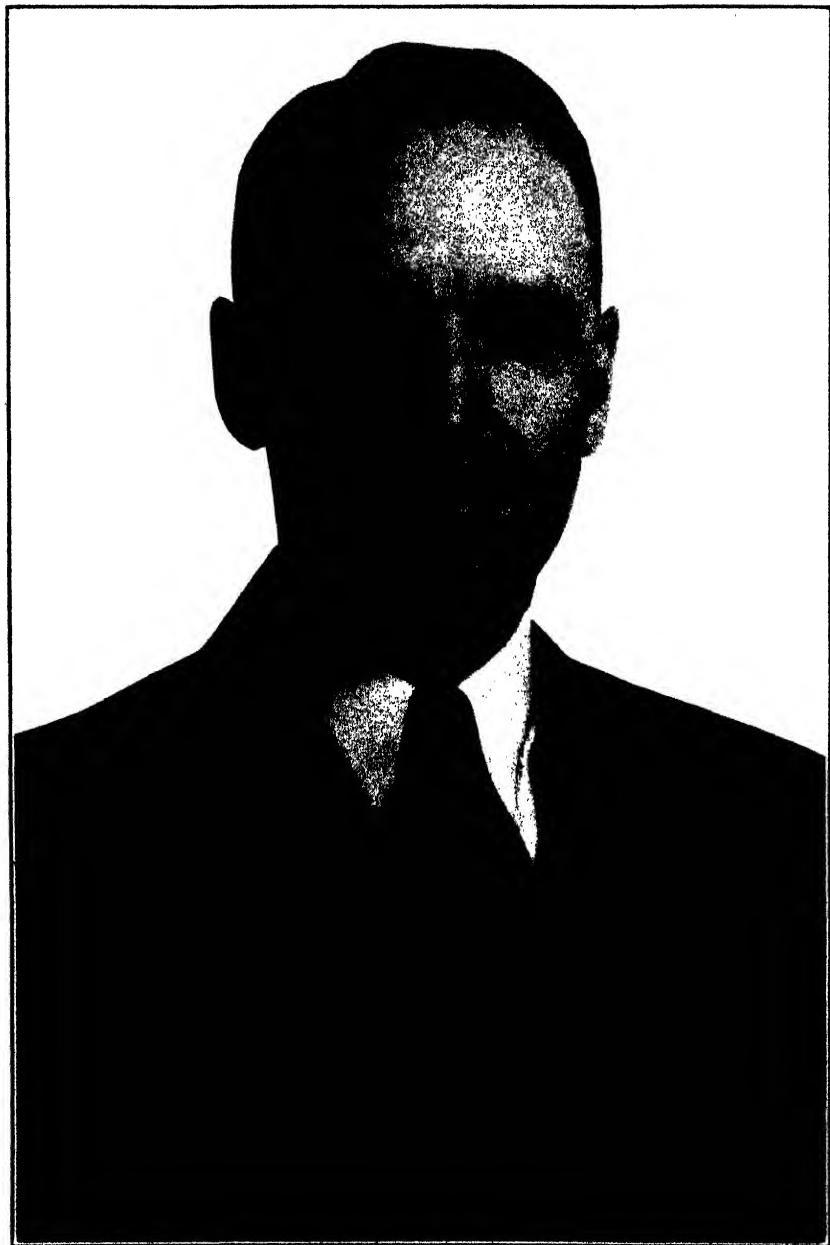
*Length of Review Articles:* The proper control of the length of Review Articles has developed into somewhat of a problem. You may recall that, upon the joint recommendation of the editor and this Committee, the Board approved in substance the following ruling on size of manuscripts acceptable for publication in the JOURNAL.

1. That manuscripts be limited, as far as possible, to twelve printed pages.
2. That the cost of pages in excess of twelve be charged to the author on the basis of \$5.00 per page.
3. That, in the case of manuscripts of unusual merit, no charge be made for pages in excess of twelve.

The above ruling was established prior to the advent of soliciting Review Articles for publication. It appears obvious from the very purpose and the consequent nature of review articles, that the above ruling on page limit should not be expected to apply to these special articles. These articles cannot be consistently limited to any such page limit, nor perhaps to any arbitrary page limit.

However, experience with unduly long review articles suggested the advisability of providing some means to avoid receipt for publication of Review Articles of unreasonable length. It is our unanimous opinion that an approximate length of 25 printed pages may well be considered ample in most cases. In addition, we feel that it may prove helpful to the author and may be welcomed by him to mention to him, at the time the subject is assigned, the JOURNAL's conception of an approximate desirable page limit.

At the same time, we consider undesirable any ruling that might embarrass authors of review articles by any set page limit, and we hold that the most effective and acceptable control of the article-size must lie in the judicious selection of the subject and of the author. It is, therefore, by



PROFESSOR H. W. CAVE, PRESIDENT-ELECT

efforts along these lines that we hope to accomplish satisfactory control of the page limit of review articles.

*Reorganization of Abstract Service:* The preparation of plans for the early reorganization of the Abstract Service is now in progress. The purpose is to make this service more complete and to improve its quality. There appears to be too much delegation of responsibility, too unwieldy an organization. We need fewer but more really conscientious abstractors.

Control on the part of those in authority has been inadequate apparently largely because of the total absence of any financial consideration for this work. Your Committee respectfully recommend, therefore, that the problem of compensating for abstract editors and abstractors be given serious consideration by the Board and that definite action be taken at this meeting.

Respectfully submitted by

*The Committee on Journal Management*

A. A. BORLAND,

R. B. STOLTZ,

O. F. HUNZIKER, *Chairman*

Mr. Ellington moved and Mr. Macy seconded that the report be accepted.

Editor T. S. Sutton was then called upon, and presented the following report:

Your Editor begs to submit the following brief report:

*1. Summary of Journal Contents.*

During the past year, July, 1939, to May, 1940, inclusive, the JOURNAL OF DAIRY SCIENCE has carried 97 papers exclusive of the proceedings of the annual meeting, announcement material, necrologies, membership and circulation lists and abstracts. These 97 papers occupied 945 pages.

A classification of these papers shows that 53 papers covering 450 pages were devoted to manufacturing, 31 papers covering 296 pages to production and 10 papers occupying 80 pages were of basic interest to both divisions. In addition, three review articles have been published occupying 119 pages, also a special report of the Students' National Contest in Judging Dairy Products covering 4 pages.

During the same period 94 pages have been devoted to announcement material, proceedings 34th annual meeting, membership lists, index, etc., and 206 pages were occupied by abstracts of literature. This is 1245 pages in all. This does not include the June, 1939, issue which contains the program and abstracts of papers.

Obviously, it has been necessary to increase the JOURNAL size somewhat in order to accommodate all accepted material without falling farther behind in publication.

*2. Origin of Contributions.*

Last year we reported the origin of the papers for the year 1938 and 1939 only. In this report we felt it would be of interest to report the origin of papers which appeared in the JOURNAL from July, 1938, to May, 1940, inclusive.

During this two-year period papers have been published originating in 28 states, the United States Department of Agriculture, Canada and Hawaii. In addition, scientific papers prepared by two American Dairy Science Association Committees have been published. The number of papers from any one state has varied from 1 to 21 for the period. The number of contributions by states is shown in the accompanying table.

CONTRIBUTIONS BY STATES; JULY 1938-MAY 1940 INCLUSIVE

State	Number Papers	State	Number Papers
New York .....	21	South Dakota .....	3
Illinois .....	18	Arizona .....	3
Wisconsin .....	16	Idaho .....	2
Michigan .....	14	West Virginia .....	2
U.S.D.A. ....	13	Canada .....	2
Iowa .....	12	Florida .....	1
Minnesota .....	11	Hawaii .....	1
Pennsylvania .....	9	Montana .....	1
Kansas .....	8	Nebraska .....	1
New Jersey .....	8	Nevada .....	1
California .....	7	North Dakota .....	1
Oklahoma .....	6	Oregon .....	1
Ohio .....	5	South Carolina .....	1
Maryland .....	5	Virginia .....	1
Indiana .....	4	Washington .....	1
Committee reports .....	4	Massachusetts .....	1
Missouri .....	4		—

28 states, U.S.D.A., Committee reports, Canada and Hawaii.

*3. Review Articles.*

During the past year three review articles have been published. A fourth has been received and is scheduled to appear in the July, 1940, issue.

The published reviews have been well received. With a little special effort on our part, we were able to sell over 1,000 extra copies of the JOURNAL in which one of these reviews appeared.

Of those originally planned for, only a few are yet to be received. In view of this, a second group of reviews have been planned. The first of these probably will not be ready for the JOURNAL until sometime this coming fall.

In extending our invitation to these reviewers, we have suggested that the subject-matter field be limited in such a way that the review will not

be too lengthy, yet we insist that the literature within this restricted subject-matter field be completely covered.

#### 4. *The Twenty-Year Index.*

The preparation of a 20-year index is under way. The author index covering the first 20 volumes has been received.

#### 5. *A Style Standard for Preparing Manuscripts.*

A committee appointed by President Guthrie is working on this important project. We trust that we will shortly have a report from this committee to serve as a helpful guide in the preparation and editing of manuscripts to be published in our JOURNAL.

#### 6. *The Abstract Section.*

In certain areas this work has been and continues to be highly commendable; in others, the work of abstracting has been neglected. A large number of those listed as abstractors have submitted no abstracts during the past year. A number of journals have not been abstracted.

The proposed plan of reorganization embodies the following points:

- a. Reorganization of the abstract editorial board.
- b. Reducing, by about one-half, the number of abstractors.
- c. Relocation of those journals not regularly abstracted. It may be advisable to drop certain journals which do not carry articles of interest to the industry.
- d. Have special publications (Experiment Station Bulletins, leaflets, circulars, etc.) mailed direct to the editor's office. These will then be submitted to the abstract editor in whose subject-matter field they lie, for abstracting.

To support and strengthen a feeling of responsibility to the JOURNAL, and as an acknowledgment of the services rendered to the JOURNAL, we recommend that the Board of Directors take favorable action on the suggestion of the Journal Management Committee regarding compensation of abstract editors and abstractors.

Again we want to take this opportunity to address a word of appreciation to all of those who have given so generously of their time and efforts in assisting with the editorial work. Without this loyal support the task of managing the editorial affairs of the JOURNAL would present an impossible problem. In particular, we wish to thank: the Journal Management Committee for their wise council and helpful suggestions, the editorial board and others who have reviewed manuscripts, and the abstract editors and abstractors for their generous contributions. To all of these and others we are grateful. To them should go the credit for any measure of success attained.

Respectfully,

T. S. SUTTON, *Editor.*

Mr. Ely moved and Mr. Parker seconded the acceptance of the report.

A letter from Mr. G. Bohstedt was read concerning our Association contributing towards the financial support of Biological Abstracts. It was moved by Mr. Macy and seconded by Mr. Ellington that the letter be referred to the Journal Management Committee for recommendation.

It was moved and seconded that the Secretary submit a proposed budget as suggested by past editor Dahlberg.

It was moved by Mr. Cave and seconded by Mr. Macy that the Eastern Division be allotted \$15.00 in addition to the usual \$25.00 to meet their expenses for this year, and that a committee of three directors be appointed to make a study and to investigate the need for our Association in supporting the Divisions, and to define the geographical limits of the Divisions and report back to the Board of Directors. The committee appointed is as follows:

J. W. LINN, *Chairman*  
E. V. ELLINGTON  
FORDYCE ELY

Upon motion, duly seconded, the meeting was adjourned to meet in this room at 4:00 p.m. on Tuesday, June 25.

MEETING OF BOARD OF DIRECTORS AMERICAN DAIRY SCIENCE ASSOCIATION  
4:00 P.M., June 25, 1940

A meeting of the Board of Directors of the American Dairy Science Association was held in the Memorial Union Building, Tuesday, June 25, 1940, at 4:00 p.m.

Present: President E. S. Guthrie; Vice-President H. W. Cave; Secretary-Treasurer R. B. Stoltz; Directors, Harold Macy, J. W. Linn, E. V. Ellington, M. E. Parker, C. N. Shepardson, Fordyce Ely.

Absent: Director, Earl Weaver.

The minutes of the Monday evening Board meeting were read and approved. The Secretary-Treasurer had previously sent a report of the Certified Public Accountant, giving an itemized list of receipts and expenditures, to each member of the Board of Directors. This report had been referred by the President to an auditing committee.

May 20, 1940

To the Members of the American Dairy Science Association  
Gentlemen:

Mr. Walter C. Burnham, of Columbus, Ohio, Certified Public Accountant, has made an audit and report of the financial condition of the Association as of January 1, 1940. The Auditing Committee has conferred with Mr. Burnham and is satisfied that he has made a careful examination of all the assets and liabilities of the Association and that all the accounts are accurate. The committee is satisfied that the

balance sheet and related summary of profit and loss fairly represents the financial condition of the American Dairy Science Association.

Respectfully submitted,

J. F. Lyman (Signed)

I. R. Krill (Signed)

W. L. Slatter (Signed)

*Auditing Committee*

*American Dairy Science Association*

Mr. Shepherdson moved and Mr. Linn seconded that the proposed budget as submitted be approved.

A communication was read from a direct advertising and sales promotion company, and upon motion by Mr. Macy and seconded by Mr. Ellington, the matter was laid on the table.

The committee, consisting of Mr. H. J. Brueckner, Chairman, and Mr. A. A. Borland, appointed by President Guthrie to study the result of the questionnaire pertaining to changing the time of the annual meeting reported as follows:

It is the opinion of your committee, as a result of studying the replies from the various institutions to the questionnaire sent by Secretary Stoltz on September 7, 1939, that the latter part of June is the most satisfactory time for holding the annual meetings of the American Dairy Science Association and that if agreeable to the host institution, the meetings be held the last week in June in order to give the men from a few of the schools a little more time to get to the meetings.

Members of Committee

A. A. BORLAND

H. J. BRUECKNER, *Chairman*

The report of the committee was accepted, and it was the consensus of opinion of the Board that they preferred the time of meeting to be held in Vermont to be the week of June 23.

The Board then adjourned to meet at 9:30 P.M.

MEETING OF BOARD OF DIRECTORS AMERICAN DAIRY SCIENCE ASSOCIATION

9:30 P.M., June 25, 1940

A meeting of the Board of Directors of the American Dairy Science Association was held in the Memorial Union Building, Tuesday, June 25, 1940, at 9:30 P.M.

Present: President E. S. Guthrie; Vice-President H. W. Cave; Secretary-Treasurer R. B. Stoltz; Directors, Harold Macy, J. D. Linn, E. V. Ellington, M. E. Parker, C. N. Shepardson, Fordyce Ely, Earl Weaver.

A petition was presented from the New York State School of Agriculture at Alfred, New York, petitioning to have a junior chapter of the American

Dairy Science Association. The Secretary was instructed to advise the School of the clause in the by-laws stating that student chapters may be formed in four-year agricultural colleges, and inasmuch as this agricultural school is a two-year course, they would be ineligible.

The Secretary was authorized to approve all other applications of student branches that conform with the regulations.

A communication was read from the Union of American Biological Societies. Upon motion duly seconded this was referred to the Journal Management Committee. The President was authorized to appoint a representative to the National Research Council.

Upon motion by Mr. Ely and seconded by Mr. Shepardson, the Secretary was instructed to enclose a suitable application blank for the Borden award in the letter that goes to the membership with ballots, the blank to contain a brief copy of the rules.

Mr. Weaver resubmitted the invitation from the Michigan State College for the Association to hold its annual meeting in 1942. Mr. Cave moved and Mr. Ellington seconded that the invitation be accepted.

Mr. Ely moved and Mr. Macy seconded that the Secretary be instructed to send the secretaries of the various divisions their allotment to spend as they see fit in the interest of the division meetings.

Mr. Shepardson moved and Mr. Weaver seconded that the President appoint a committee from the Board, one Board member to represent each of the three sections to go before the business meeting of the section on Wednesday afternoon at 4:00 P.M., and discuss with the sections the policies of their program, and request that the sections take action and appoint a rotating program committee to meet with the Board of Directors at 6:00 P.M. Wednesday. The following Board members were appointed: Mr. Cave to meet with the Production Section; Mr. Shepardson, the Extension Section; and Mr. Parker, the Manufacturing Section.

Upon motion duly seconded the Secretary was authorized to submit an amendment to our by-laws at the meeting Thursday afternoon at 4:00 P.M., changing the necessary attendance to make a quorum from ten per cent to five per cent.

Upon motion duly seconded the meeting was adjourned to meet again Wednesday at 6:00 P.M., the place to be designated on the bulletin board.

#### MEETING OF BOARD OF DIRECTORS AMERICAN DAIRY SCIENCE ASSOCIATION

6:00 P.M., June 26, 1940

A meeting of the Board of Directors of the American Dairy Science Association was held in the Memorial Union Building, Wednesday, June 26, 1940, at 6:00 P.M.

Present: President E. S. Guthrie; Vice-President H. W. Cave; Secretary-

Treasurer R. B. Stoltz; Directors, Harold Macy, J. W. Linn, E. V. Ellington, M. E. Parker, C. N. Shepardson, Fordyce Ely.

Absent: Director, Earl Weaver.

Invited Guests: Extension Section—O. J. Hill, G. E. Vergeront. Production Section—G. H. Wise, Glenn Salisbury and W. E. Petersen. Manufacturing Section—B. E. Horrall, P. A. Downs, P. S. Lucas. Also J. A. Nelson, H. B. Ellenberger, and H. W. Gregory.

Mr. Macy moved and Mr. Parker seconded that it be the recommendation of the Board that the program committee arrange for a program providing for:—

- (1) symposia
- (2) the presentation of original papers
- (3) committee reports

Mr. Macy moved and Mr. Ellington seconded that it be recommended by the Board that the general program committee consist of the Retiring President, acting as chairman, and the chairman of the other three sectional program committees.

The Board then adjourned.

#### MEETING OF BOARD OF DIRECTORS AMERICAN DAIRY SCIENCE ASSOCIATION

5:00 P.M., June 27, 1940

A meeting of the Board of Directors of the American Dairy Science Association was held in the Memorial Union Building, Thursday, June 27, 1940, at 5:00 p.m.

Present: President E. S. Guthrie; Vice-President H. W. Cave; Secretary-Treasurer R. B. Stoltz; Directors, Harold Macy, J. W. Linn, E. V. Ellington, M. E. Parker, C. N. Shepardson.

Absent: Directors, Fordyce Ely and Earl Weaver.

The previous minutes of the Board of Directors were read and approved.

Mr. Shepardson moved and Mr. Parker seconded that the host institution and the division and sections be furnished Association letterheads for their official business.

Upon motion duly seconded the Board then adjourned.

AMERICAN DAIRY SCIENCE ASSOCIATION PRESENTED  
BORDEN AWARDS TO B. W. HAMMER AND C. W.  
TURNER

*at the*

ANNUAL BANQUET

*Purdue Memorial Union Building*  
*West Lafayette, Indiana, June 27, 1940*

H. W. Gregory, toastmaster, made the following statement:

"Since 1936 we have had an award, which the American Dairy Science Association has prized very highly. The Borden Company, recognizing the valuable work of those working in the field of dairy science, has offered to the Association two annual awards of \$1,000 in cash and a medal for meritorious scientific work in the field of dairy science. These awards are prized highly by the Dairy Science Association. We have a representative of the Borden Company here with us, and I would just like at this point for him to stand, and I will introduce him as W. A. Wentworth, of the Borden Company. It may be well for me to state the requirements for those who are eligible for this award. I would like to introduce the man who will present the recipient for the manufacturing award."

Mr. N. W. Hepburn:

"In the study of almost any important industrial development it early becomes apparent that practically all notable advancements in the field under observation can be traced to some individual working in a laboratory, with characteristic modesty, and often in relative obscurity.

"These findings become tools in industry's daily operations and sometimes little thought is given to their origin. It is indeed gratifying to find in our dairy industry, whose progress is marked with a similar pattern, an outstanding organization ready and willing to acknowledge indebtedness to individuals whose work has contributed measurably to its growth and success. The Borden Company has caught the vision of science's relation to industry and by means of its annual awards extends recognition for work well done. On this occasion, I think it fitting that we express our appreciation for the stimulus to research in dairying that is in this manner given.

"The American Dairy Science Association, on this, the thirty-fifth annual meeting very properly devotes a general session to commemorating the work of one of America's great dairy scientists, the late Dr. Babcock, who is remembered not alone for the contributions he made but as much for the inspiration he gave to his contemporaries and those who followed after him.

"Speaking of contributions and inspiration, tonight we are happy to participate in a program of giving special recognition to another beloved dairy



B. W. HAMMER

scientist—one who has fully earned the affection in which he is held—by his work, by his character, and by the leadership and inspiration he has given. The list of students who have made great strides—and particularly those who have received their Ph.D. degrees—under his leadership, is impressive, and we who have the good fortune to be counted among his friends cannot fail to realize that, with these great groups he has shared his lovable character and his inspiration as teacher and counselor. May his good work go on.

"Now we have come to the time, Mr. Wentworth, when it is my great pleasure to present to you, in behalf of the Committee on Awards, of the American Dairy Science Association, Dr. B. W. Hammer, to receive the 1940 Borden award in Dairy Manufacturing.

"The rules of the American Dairy Science Association require that the award be given for outstanding research in the field of dairy manufactures, the results of which have been published during the preceding five-year period.

"The list of candidates placed before the members of the Dairy Manufacturing Award Committee was impressive, indeed, and the Committee gave all the most careful consideration. Dr. Hammer, of the Iowa State College, was unanimously chosen for this honor.

"Dr. Hammer was born October 7, 1886, at Hillsboro, Wisconsin. After graduation from the Hillsboro High School in 1904 he entered the University of Wisconsin, graduating with the B.S.A. degree in 1908. He continued his studies at the University of Wisconsin under Dr. E. G. Hastings and was assistant in agricultural bacteriology in 1908-1909. He was then appointed bacteriologist, associated with Dr. M. P. Ravenel at the Wisconsin State Hygienic Laboratory, at the University of Wisconsin, from 1909-1911, after which he joined the Department of Dairy Industry at Iowa State College in 1911 and has since 1916 been chief in dairy bacteriology of the Iowa Agricultural Experiment Station and Professor of Dairy Bacteriology.

"Dr. Hammer was selected for this honor on his contributions including studies on the flavor and aroma constituents of butter and cheese, factors involved in the deterioration of butter, factors involved in the ripening of various cheeses, improvement in the methods for the manufacturing and curing of cheese, classification of microorganisms important in dairy products, action of various microorganisms on dairy products and development of new laboratory methods.

"Dr. Hammer's research has been both fundamental and practical. It is known, not merely throughout the United States, but in any country where dairying has been developed. Dr. Hammer is not merely known for his research—he is also recognized as one of the outstanding teachers in the Dairy Industry field and his textbook on dairy bacteriology is the

standard textbook not only in the United States, but also in many foreign countries.

Mr. Wentworth, I take great pleasure in presenting Dr. B. W. Hamner to you for the Borden Award."

Mr. W. A. Wentworth:

"Dr. Hammer, it gives me a great deal of pleasure to present to you this award on behalf of the Borden Company, and with it goes a slip of paper, which I am deeply happy to present."

Mr. Gregory, the toastmaster, then introduced Mr. S. J. Bechdel, of Pennsylvania State College, Chairman of the Production Awards Committee, who presented the recipient for the production award:

"In accordance with the regulations governing the Borden Awards adopted by the Board of Directors as reported in the August, 1939, issue of the JOURNAL OF DAIRY SCIENCE, it is the duty of the Award Committee in Dairy Production to select the person to be the recipient this year. The three members of this Committee gave careful consideration to the credentials of the nominees submitted by the Nominating Committee and have unanimously chosen Doctor Charles Wesley Turner, Professor of Dairy Husbandry, University of Missouri, Columbia, Missouri, to be honored with the award. The choice of Doctor Turner is based upon his outstanding and extensive fundamental research on the physiology and anatomy of the mammary gland. His fundamental work on the endocrinology of milk secretion has established new facts in this field and as a result he is receiving world-wide recognition. Although his research work along these lines has covered a twelve year period, the Committee would call attention to the fact that a total of 52 scientific papers, 10 scientific bulletins, 2 books, and 5 popular bulletins have been published under the authorship of Doctor Turner and his colleagues within the last five years. He has brought honor to himself and to the American Dairy Science Association through his untiring efforts to serve the industry in the field of Dairy Production. Mr. Wentworth, it is a real pleasure to present to you Doctor Charles Wesley Turner as the recipient of the Borden Award in Dairy Production."

Mr. Wentworth then said:

"Dr. Turner, may I again present this award on behalf of the Borden Company and the American Dairy Science Association, commending you on the work that you have done, and as I have said before, with it goes this slip of paper.

"Mr. Chairman, members of the American Dairy Science Association, and ladies and guests. This is the fourth year that this Association has given recognition to those in your ranks of your membership, who have done such meritorious work in research that you have seen fit to name them as



C. W. TURNER

the outstanding men of the year. I am sure that it is considered a privilege on the part of the Bordon Company to join with you on that type of recognition. The company feels, as I know you do, and as has been said formerly here by speakers, that this industry is permanently founded when research is at its best. Tonight you have added to those names who have previously been given this award. I want to mention those other names, who have previously received it so that perhaps it will be fresh in your mind.

1937—Dr. L. A. Rogers and Dr. C. F. Huffman

1938—Dr. K. G. Weekel and Dr. W. E. Krauss

1939—Dr. S. L. Tuckey and Dr. R. E. Hodgson

"So you men of the American Dairy Science Association have named eight men, who are outstanding in the field of research in dairying. Let those men and those names be an inspiration to the rest of you and to the rest who have come in the recent years to do in this industry so that it may better serve the welfare of the producers and the welfare of consumers in this great land. I thank you."



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## LIPOLYTIC ACTIVITY IN MILK AND CREAM

D. C. ROAIHEN AND H. H. SOMMER

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Lipase in milk was first convincingly demonstrated in work reported by Maass (1) in 1909. Sterilized cream, with formalin as a preservative, on inoculation with raw cream, showed increases in acidity on incubation, with the increases proportional to the inoculum. Greater increases were caused by cream from milk late in the lactation period. However, as late as 1922 Palmer (2) failed to demonstrate the presence of lipase in milk, but Rice and Markley (3), reporting in the same journal issue, demonstrated lipase by the increase in acidity that developed in a substrate of boiled cream, saturated with sugar, and inoculated with raw milk. Later in the same year Palmer (4) reported lipase in milk taken near the end of the lactation period, and attributed the development of a bitter flavor and rancid odor in such milk mainly to butyric acid set free by lipolysis. Since then the presence of lipase in milk has been generally recognized, and many interesting facts have been reported concerning its activity.

In studying the lipolytic activity of milk various procedures have been used. Early methods have been reviewed by Rice and Markley. In the early work various preservatives, including chloroform and formaldehyde, were used to exclude bacterial action, but chloroform was found to retard lipase action (5), and formaldehyde also limits it. The finding that formaldehyde limits lipolysis to various extents in different milk samples led Herrington and Krukovsky (6) to postulate at least two lipases in milk. In more recent work chemical preservatives have been avoided by using sugar-saturated-cream as the substrate (3), or by making the observations on the milk or cream itself, allowing lipolysis to proceed at low temperatures to limit bacterial activity (6), or by using a buffer substrate containing tri-butyrin (7). The extent of lipolysis has usually been measured by titration with standard alkali, the increase in titer being attributed to free fatty acids. The titrations have been applied to the sample directly, with or without the addition of organic fat solvents to make the free fatty acids more accessible, or to the fat obtained from the sample by churning and oiling-off the resulting butter (6), or to the steam distillate from the sample (7). The decrease

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in surface tension resulting from lipolysis in milk has been suggested as a measure (8).

The view has persisted that lipolytic activity in milk is greatest near the end of the lactation period. Since the work of Maass (1) and Palmer (4) various reports have tended to confirm this view. Sharp and DeTomasi (9) in reporting on lipolysis in raw cream adopted this view. Hileman and Courtney (10) attributed the maximum lipolytic activity in December and January in part to the lactation cycle. Krukovsky and Sharp (11) attributed the slow churning of cream from cows in advanced lactation to lipolytic action. Yet studies of this factor have failed to establish a close relationship between lipase content and the lactation period. Mattick and Kay (7), working with a buffered tributyrin substrate, failed to find a higher lipase (butyryinase) content in advanced lactation. Pfeffer, Jackson and Weckel (12), working with sugar-saturated-cream substrate, failed to find a relationship between lipase content and the stage of lactation. Even when the fat in the milk sample itself served as the substrate, thereby involving differences in fat content, fat globule sizes and surface area of the fat, Herrington and Krukovsky (6) found no relationship between lipolysis and the stage of lactation, stage of gestation, or the amount of milk produced by each cow.

The study of the lipase content of milk is complicated by the factors that greatly alter the activity of the lipase that is present. Dorner and Widmer (13) found that homogenization of raw milk greatly increased subsequent lipolysis. This has been confirmed repeatedly and is generally assumed to be due to the increased area of contact between the fat and the aqueous phase containing the lipase, but may possibly involve other factors (12). Krukovsky and Sharp (14) have reviewed the literature pertaining to activation of lipase by shaking and have confirmed and extended it to show that shaking is particularly effective when the fat is in the liquid state. Herrington and Krukovsky (6) found less lipolysis in milk that was cooled rapidly by means of a tubular cooler as compared with the same milk cooled in air at 0° C. or in water at 0° C. They also found that precooling followed by warming activated the lipase as subsequently observed at lower temperatures. The effectiveness of the precooling increased as the temperature was lowered from 25° C. to 0° C., but the duration of the precooled condition seemed to be of little importance. The effectiveness of the warming after the precooling increased up to 30° C. and beyond this the effect decreased so that at about 37° C. the effect of the precooling was undone. The earlier finding by Sharp and DeTomasi (9) that less lipolysis takes place in cream from milk forewarmed to 110°–115° F. and separated at 75°–85° F. than in cream from the same milk forewarmed and separated at 75°–85° F., might be due to these activation factors. Davies (14) found that lipolysis was inhibited by heavy metals, especially copper. Herrington and Krukovsky (6) found that 0.2 and 0.4 p.p.m. of copper reduced lipolysis at 0° C. about 20 per cent.

#### METHOD

In this study lipolysis was observed in (a) samples of milk and cream held in a refrigerator (near 0° C.), and (b) sugar-saturated-cream inoculated with samples of skim milk, milk or cream, and held at 37° C. The sugar-saturated-cream was prepared from 40 per cent cream by adding 2 parts of sucrose to every 1 part of water naturally present in the cream, heating to 145° F., homogenizing at 1,500 lb. pressure, portioning the product into bottles, and sterilizing in streaming steam for 30 minutes. In this manner the substrate could be prepared and kept in batches of sufficient size to serve several series of experiments.

Lipolysis was followed by determining the titer of the steam distillate at the start and after holding. For this purpose 20 grams of the sample were acidified with 1.2 cc. N/1 sulphuric acid and then subjected to steam distillation, collecting 200 cc. of distillate in a closed receiving vessel fitted with a soda lime tube. This distillate was titrated with N/50 sodium hydroxide from a microburette using 5 drops of 1 per cent phenolphthalein as indicator. The following precautions were found essential: Use glass equipment fitted throughout with glass joints. Generate the steam from distilled water, and before placing the 20 grams of the sample in the distilling flask, free the water and the equipment from carbon dioxide by distilling over at least 150 cc. of distillate. Keep the distilling rate reasonably uniform, and keep the volume of the sample substantially constant by placing a small flame under the distilling flask. With these precautions duplicate determinations consistently checked within 0.03 cc.

While this procedure measures only the volatile, soluble acids, the results are comparable in a series of samples where the fat is the same. In the case of the sugar-saturated-cream experiments the fats differed only to the slight extent that fat was introduced in the inoculum.

In experiments reported in tables 2 and 5, where cream samples were held at refrigerator temperature, fat samples were obtained by churning and oiling-off for a comparison of the acid number of the fat by the Herrington-Krukovsky procedure with the titer of the steam distillate from the cream samples. The results were quite closely parallel.

#### EXPERIMENTAL

##### *The Effect of Tributyrin and Formaldehyde on Lipolysis*

As a check on the method of procedure here adopted and on the results reported in the literature, the sugar-saturated-cream substrate was inoculated with 40 per cent raw cream, without and with further additions of tributyrin and formaldehyde as shown in table 1.

Formaldehyde limited lipolysis to about a third or a half, and tributyrin greatly increased lipolysis as measured by the titer of the steam distillate. In both cases no distinction was apparent between the two levels of addition.

TABLE 1  
*The effect of tributyrin and formaldehyde on lipolysis*

Sample 100 grams substrate inoculated with 10 grams 40% raw cream plus	Acidity of steam distillate, cc. N/50 NaOH, after incubation at 37° C. for			
	0 hr.	24 hr.	48 hr.	96 hr.
Control, no further additions .....	0.50	2.76	4.00	5.90
Formalin,* 0.05 cc. ....	0.49	1.35	1.47	1.79
Formalin, 0.15 cc. ....	0.50	1.37	1.49	1.77
Tributyrin, 1 gram .....	1.51	10.27	16.33	18.69
Tributyrin, 2 grams .....	2.23	10.27	16.33	18.69
Control, no further additions .....	0.38	2.31	3.87	4.99
Formalin, 0.15 cc. ....	0.38	1.16	1.45	1.80
Tributyrin, 1 gram .....	1.69	9.43	15.21	18.00
Control, no further additions .....	0.46	5.41	6.63	7.01
Formalin, 0.15 cc. ....	0.46	1.86	2.61	2.93
Tributyrin, 1 gram .....	1.96	12.60	17.17	21.03

\* Containing 37 per cent formaldehyde.

#### *A Comparison of Machine Separated and Gravity Separated Cream*

Since the lipase is present in the aqueous plasma of cream but may be associated with the fat to a greater or lesser extent by adsorption, it was of interest to compare lipolysis in separator cream and gravity cream from the same original milk. The extreme difference in the forces applied in the two methods of separating the cream from the milk might affect the distribution of the lipase.

The milk and cream used in this experiment represents mixed milk from a number of dairies as received for the commercial operations of the University Creamery. With the milk forewarmed to 30–35° C. in the commercial separating operations, cream and skim milk were obtained simultaneously and at the same moment some of the same milk, but not forewarmed, was taken for gravity creaming. These samples were obtained in the morning, were cooled to and held at 2–3° C. until mid-afternoon when the gravity cream was removed from the milk. The gravity cream was tested for fat (usually about 20 per cent fat) and the separator cream in each comparison was standardized to the same fat content by adding the proper amount of the separator skim milk. A portion of the standardized, separator cream was pasteurized at 145° F. for 30 minutes to serve as a control. The three samples,—pasteurized cream, separator cream and gravity cream, were held at 2–3° C. for lipolysis observations by two methods: (a) by determining the titer of the steam distillate from 20 grams of the cream, and (b) by churning the cream and determining the acid number of the fat by the method of Herrington and Krukovsky (6). Eight such comparisons were made, and in each case the gravity cream showed significantly greater lipolysis than the separator cream in spite of the fact that the separator cream was favored by

the activating effect of forewarming while the gravity cream was not. Typical results are given in table 2.

TABLE 2  
*A comparison of machine separated and gravity separated creams*

Sample	Acid number* of fat after holding at 2-3° C. for		Acidity of distillate after holding at 2-3° C. for	
	0 hr.	48 hr.	0 hr.	48 hr.
Control (pasteurized cream)	0.38	0.40	0.69	0.71
Separator cream	0.38	0.60	0.69	8.63
Gravity cream	0.38	0.68	0.71	9.11
Control (pasteurized cream)	0.40	0.40	0.58	0.61
Separator cream	0.40	0.56	0.57	7.49
Gravity cream	0.40	0.61	0.60	9.36
Control (pasteurized cream)	0.36	0.36	0.71	0.71
Separator cream	0.36	0.60	0.71	8.37
Gravity cream	0.38	0.69	0.74	11.29
Control (pasteurized cream)	0.34	0.35	0.87	0.89
Separator cream	0.34	0.58	0.87	6.83
Gravity cream	0.33	0.63	0.91	9.12

\* Ce. of N/20 NaOH to titrate the acidity in 5 grams of fat.

† Ce. of N/50 NaOH to titrate the steam distillate from 20 grams of cream.

### *The Effect of Shaking on Lipolysis*

The effect of shaking on lipolysis was observed on samples of milk, on samples of the same milk in sugar-saturated-cream, and on samples of cream and corresponding skim milk in sugar-saturated-cream. All of these samples, including those prepared with the sugar-saturated-cream substrate, were held at 3-4° C. except during the shaking period, when all of them including the controls (unshaken), were brought to 25° C. The shaken samples were shaken during the first three hours of each 24-hour period in a mechanical shaker in which they travelled horizontally in a path 1½ inches long at the rate of 195 strokes (complete cycles) per min. Lipolysis was measured by the steam distillation and titration procedure applied at 0, 24, 48, and 96 hours. The results are given in table 3.

Where the milk samples without additions were held, shaking increased lipolysis, but where the milk, cream or skim milk was used to inoculate the sugar-saturated-cream substrate, shaking caused a decrease. In the former case the increase was apparently due to washing the fat surface free from end products; increased fat surface due to dispersion of the fat could hardly be involved since the conditions were conducive to churning. In the inoculated substrate this apparently is not a limiting factor, and shaking then produces the detrimental effect that is quite common for enzyme action.

TABLE 3  
*The effect of shaking on lipolysis*

Sample	Acidity of steam distillate in cc. of N/50 NaOH after holding* time of			
	0 hr.	24 hr.	48 hr.	96 hr.
Whole milk No. 1	1.22-1.22†	2.27-3.22	3.26-5.05	4.16-6.69
Whole milk No. 2	1.72-1.72	1.91-2.72	2.22-3.07	2.42-3.57
Whole milk No. 3	1.46-1.46	1.80-2.76	2.26-3.97	2.59-4.60
Whole milk No. 4	1.33-1.33	2.35-2.41	3.88-4.08	4.26-5.69
Whole milk No. 5	1.41-1.41	2.43-2.99	4.03-4.47	4.69-5.99
Whole milk No. 6	1.09-1.08	2.17-2.81	3.64-4.00	4.19-5.71
Whole milk No. 7	1.26-1.26	2.22-2.93	3.67-4.20	4.39-5.91
100 grams of sugar-saturated-cream plus 10 grams of:				
Whole milk No. 4	1.89-1.89	2.43-2.36	3.91-3.42	5.64-4.63
Whole milk No. 5	1.91-1.91	2.78-2.65	4.12-3.68	6.94-5.78
Whole milk No. 6	1.80-1.80	2.31-2.19	3.45-2.97	6.44-5.23
Whole milk No. 7	1.83-1.83	2.75-2.70	3.54-2.75	6.36-5.39
Whole milk No. 8	1.84-1.84	2.44-2.29	3.30-2.98	5.63-4.77
110° F. cream from No. 8‡	2.04-2.04	2.76-2.59	3.34-3.04	4.78-4.77
110° F. skim milk from No. 8	1.93-1.93	2.59-2.40	3.11-2.97	5.18-4.56
75° F. cream from No. 8	1.80-1.80	2.51-2.41	3.44-3.27	5.22-4.85
75° F. skim milk from No. 8	1.84-1.84	2.63-2.30	3.38-2.85	5.62-4.91

\* The holding temperature was 3-4° C. except during the first 3 hours of every 24-hour period when the temperature was 25° C.

† In these pairs of figures the first is the value found for the unshaken sample, the second for the corresponding shaken sample.

‡ The temperature here refers to the temperature of the milk at the time of separation.

#### *The Effect of Temperature on Lipolysis*

To observe the effect of the several temperature levels used in this and other work, sugar-saturated-cream was inoculated with whole milk in the proportion of 10 to 1 and held at refrigerator (3-4° C.), room (27° C.), and incubator (37° C.) temperatures. At 0, 24, 48, and 96 hours, portions were steam distilled and the distillate titrated. Duplicate determinations in this case were made by using 2 samples at each temperature with the samples

TABLE 4  
*The effect of temperature on lipolysis*

Temperature °C.	Acidity of the steam distillate in cc. of N/50 NaOH after holding at the specified temperature for			
	0 hr.	24 hr.	48 hr.	96 hr.
3-4	0.83-0.83*	3.42-3.54	4.05- 4.22	4.26- 4.31
27	0.83-0.84	3.85-4.13	8.81- 9.28	9.78-10.10
37	0.83-0.83	4.04-4.66	10.67-11.01	12.08-12.31
3-4	0.69-0.69	2.78-2.79	3.47- 3.22	3.91- 3.91
27	0.69-0.69	3.11-3.01	8.01- 7.88	10.10- 9.89
37	0.68-0.69	3.64-3.56	9.16- 8.97	12.49-12.38

\* Paired figures represent duplicate determinations but with different batches of substrate (batches 1 and 2 in the first series, batches 1 and 3 in the second).

prepared from different batches of substrate but inoculated with the same milk throughout. The experiment was repeated using one of the former batches of substrate and a new batch. The results are given in table 4.

The results were in harmony with expectations; lipolysis was most extensive at 37° C., slightly less at 27° C. and decidedly less at 3-4° C. in the case of 48 and 96 hour holding. However, the difference after only 24 hours holding was surprisingly small. A slight difference in the duplicate determinations is attributable to the substrates; in the first series batch 1 consistently yielded lower results than batch 2, and in the second series, batch 1 yielded higher results than batch 3 in practically all cases.

#### *The Effect of pH on Lipolysis*

To observe the effect of hydrogen ion concentration on the rate of lipolysis a series of samples, ranging from about pH 6.2 to 9.0, were prepared from 40 per cent raw cream with appropriate additions of N/1 lactic acid or N/1 sodium hydroxide as required. The samples were held at 3-4° C. for 48 hours. At 0 and 48 hours the pH of the samples was determined at room temperature by means of the quinhydrone electrode, and 20 gram portions were steam distilled and the distillate titrated with N/50 NaOH. The remainder of the cream at 48 hours was churned and the fat used to determine its acid number by the Herrington and Krukovsky procedure. The results from a number of such series indicate that the optimum pH is at pH 8.4 to 8.6. Typical results are given in table 5.

TABLE 5  
*The effect of pH on lipolysis*

Sample	pH after holding at 3-4° C. for		Acidity of distillate in cc. N/50 NaOH after		Acid No. of the fat after
	0 hr.	48 hr.	0 hr.	48 hr.	
1	6.19	6.19	0.46	0.69	0.60
2	6.25	6.23	0.46	0.81	0.75
3*	6.61	6.58	0.46	2.14	1.20
4	6.81	6.62	0.46	2.84	1.85
5	7.01	6.69	0.46	3.11	1.98
6	7.14	6.64	0.46	3.37	2.36
7	7.15	6.50	0.46	4.54	2.84
8	7.38	6.70	0.46	4.93	3.00
9	7.85	7.02	0.46	5.30	3.19
10	8.31	7.32	0.46	6.26	3.76
11	8.50	7.07	0.45	7.37	4.28
12	8.91	7.90	0.43	6.19	3.79

\* Cream without added acid or alkali.

#### *Lipase Content of Milk from Individual Cows*

Representative samples of milk (afternoon milking) were taken from individual cows of the University herd, and were promptly cooled to 3-4° C.

Lipolysis observations were made on (a) sugar-saturated-cream inoculated with the milk in the proportion of 10 to 1 and incubated at 37° C., and (b) the milk samples themselves held at 3-4° C. In both cases lipolysis was followed by the titer of the steam distillate after 0, 24, and 72 hours. Another milk sample was obtained from each of the same cows about a week later and similarly examined. Table 6 gives the results arranged according to breed and the stage of lactation.

TABLE 6  
*Lipolysis in milk from individual cows*

Milk sample, breed, cow no. and mo. of lactation	Titer of steam distillate in cc. N/50 NaOH from					
	Inoculated substrate at 37° C.			Milk itself at 3-4° C.		
	0 hr.	24 hr.	72 hr.	0 hr.	24 hr.	72 hr.
Guernsey 470—2½ mo. ....	0.49*	0.74	0.89	0.79	1.13	1.56
" 445—4 " ....	0.53*	1.55	1.96	0.83	1.23	1.61
" 412—7 " ....	0.50	0.70	0.84	0.64	1.16	1.62
" 427—7 " ....	0.53	1.56	1.88	0.68	1.19	1.57
" 438—10 " ....	0.49	2.14	3.51	0.50	2.07	3.42
" 432—10 " ....	0.53	3.16	4.03	0.46	2.80	3.92
Holstein 93—2½ " ....	0.49	1.91	2.23	0.47	1.74	3.04
" 98—1½ " ....	0.47	1.27	1.97	0.52	0.94	1.32
" 33—6 " ....	0.49	1.32	2.01	0.64	0.91	1.18
" 67—7½ " ....	0.48	1.71	2.25	0.56	1.27	1.94
" 91—8½ " ....	0.49	1.93	3.27	0.51	2.36	3.97
" 83—18½ " ....	0.49	2.23	3.32	0.58	2.61	3.44
" 656—3 " ....	0.49	1.29	2.16	0.49	1.29	2.83
" 660—7 " ....	0.46	1.61	2.46	0.51	2.02	2.99
" 621—10 " ....	0.47	1.37	3.31	0.62	1.36	3.61
" 809—6 " ....	0.47	2.28	3.39	0.64	2.52	3.46
Br. Swiss 622—1 " ....	0.49	1.25	2.08	0.54	1.44	2.79
" 823—10 " ....	0.47	1.58	2.35	0.64	1.68	2.18
" 660—7 " ....	0.44	0.59	1.23	0.46	1.33	2.01
" 621—10 " ....	0.44	0.51	1.01	0.43	1.00	1.83
" 809—6 " ....	0.44	0.59	1.40	0.49	2.02	5.09
" 823—10 " ....	0.46	1.47	2.42	0.62	1.86	3.29
" 809—6 " ....	0.41	1.72	2.44	0.56	1.33	4.07
" 823—10 " ....	0.46	1.01	1.58	0.77	1.00	1.04
" 823—10 " ....	0.41	0.87	1.50	0.67	0.79	1.27
" 823—10 " ....	0.46	1.19	1.80	0.88	1.31	2.16
" 823—10 " ....	0.40	1.53	1.94	0.61	1.78	2.28

\* Paired figures do not represent duplicate determinations, but rather two separate determinations on milk samples taken at intervals of about 1 week.

These results indicate appreciable variation in the lipase content or lipolytic activity in the milk from the same cow, as well as wide differences from cow to cow. In several instances the results tend to support the hypothesis that high lipolytic activity accompanies advanced lactation, but taken as a whole the data do not warrant this conclusion. No tests were made in which the lipolytic activity of milk from the same cow was followed throughout the lactation period.

#### *Relation of Temperature of Separation to Lipolysis*

To study the temperature of separation as a factor in lipolysis in cream, milk as received commercially was separated at 75° F. and at 110° F., by means of an air-tight separator in such a manner that the fat content of the two creams was alike within 0.5 per cent. Lipolysis was followed in two ways: (a) by inoculating sugar-saturated-cream in the proportion of 10 to 1 with each of the creams and skim milk separately and incubating at 37° C., and (b) by holding the cream at 3-4° C. In both cases lipolysis was measured at 0, 24, 48, and 96 hours by determining the titer of the steam distillate. See table 7.

TABLE 7  
*Temperature of separation in relation to lipolysis*

Description of sample	Acidity of steam distillate in cc. N/50 NaOH after			
	0 hr.	24 hr.	48 hr.	96 hr.
75° F. cream in substrate at 37° C.	0.47	1.81	2.67	3.11
110° F. cream " " " "	0.47	1.67	1.83	2.17
75° F. skim milk " " " "	0.47	2.23	2.98	3.48
110° F. skim milk " " " "	0.47	2.10	2.40	2.74
75° F. cream at 3-4° C.	0.89	1.60	2.66	3.00
110° F. cream at 3-4° C.	0.78	1.24	1.81	2.01
75° F. cream in substrate at 37° C.	0.47	2.00	3.27	4.65
110° F. cream " " " "	0.46	1.89	2.97	3.56
75° F. skim milk " " " "	0.46	2.15	3.85	4.91
110° F. skim milk " " " "	0.46	1.93	3.04	3.88
75° F. cream at 3-4° C.	0.75	2.99	3.26	4.01
110° F. cream at 3-4° C.	0.68	1.73	2.33	2.92
75° F. cream in substrate at 37° C.	0.52	1.64	2.39	3.11
110° F. cream " " " "	0.52	1.45	1.67	1.97
75° F. skim milk " " " "	0.52	1.67	2.43	3.26
110° F. skim milk " " " "	0.51	1.54	1.79	2.03
75° F. cream at 3-4° C.	0.76	1.19	1.87	2.16
110° F. cream at 3-4° C.	0.71	1.06	1.24	1.43

In all three experiments the 110° F. cream showed definitely less lipolysis than the 75° F. cream by both methods. This is in harmony with the findings of Sharp and DeTomasi (9). However, the 110° F. skim milk also showed definitely less lipolysis than the 75° F. skim milk. Since the milk

had been cooled (on farms) before separating, the greater lipolysis in the 75° F. cream when held at 3-4° C. might be attributed to the activation of the lipase by precooling followed by limited warming, a factor as reported by Herrington and Krukovsky (6), but the fact that the same results were obtained when the creams were inoculated into sugar-saturated-cream and incubated at 37° C. argues against this explanation, since 37° C. presumably undoes such activation. The fact that both the 110° F. cream and the 110° F. skim milk caused less lipolysis suggests that partial destruction of the lipase takes place at the higher temperature under the conditions involved in centrifugal separation, or greater loss of lipase in the separator slime (12).

#### SUMMARY

1. Formaldehyde addition to sugar-saturated-cream inoculated with raw cream greatly limited lipolysis. Tributyrin greatly increased lipolysis as here measured.
2. Gravity separated cream was found to show appreciably greater lipolytic activity than separator cream of the same fat content.
3. Shaking increased lipolysis in the case of milk samples held as such, but decreased lipolysis in the case of sugar-saturated-cream inoculated with the same milk samples. The latter effect was also observed where cream was used as the inoculum.
4. In a comparison of 3-4° C., 27° C. and 37° C., lipolysis was most extensive at 37° C., slightly less at 27° C. and decidedly less at 3-4° C.
5. The optimum pH for lipolysis in cream samples at 3-4° C. was found to be pH 8.4 to 8.6.
6. The indications are that lipolysis varies considerably in the milk from the same cow, and differs widely from cow to cow. A study of milks from 18 cows at known stages of lactation failed to indicate a relationship between lipolysis and stage of lactation.
7. Cream separated at 110° F. showed less lipolysis than cream separated at 75° F. both as observed by holding the samples at 3-4° C. and by using the cream to inoculate sugar-saturated-cream and incubating at 37° C. Similarly the 110° F. skim milk caused less lipolysis than the 75° F. skim milk in sugar-saturated-cream.

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# THE EFFECT OF HOLDER AND FLASH PASTEURIZATION ON SOME FLAVORS OF MILK. I. THE EFFECT ON MISCEL- LANEOUS FLAVORS COMMON TO COMMERCIAL RAW MILK<sup>1</sup>

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Feed has long been recognized as a possible contributing factor to the flavor of milk. Early studies on the effects of feeds and feeding practices have aided materially in the establishment of feeding rules which, when followed, result in a minimum of feed flavors in the milk produced. However, much feed-flavored milk is yet produced during certain seasons of the year. Milk distributors often reject feed-flavored milk at the receiving platform on the presumption that if such milk were mixed and processed with the other normal flavor milk, the resulting bottled product would be lowered in flavor quality if not definitely off flavor.

The purpose of this study was to determine the effect of various methods of pasteurization upon the flavor and score of the processed milk as compared to the flavor and score of the control samples. Particularly was information desired on the effects of pasteurization upon some feed flavors in order to determine if those flavors frequently resulting from the feeding of clean wholesome feeds, such as alfalfa and corn silage, were seriously objectionable to the market milk supply.

Several investigators have reported the effects of pasteurization on some flavors of a feedy or barny nature. Tracy and Ruehe (3), holder pasteurizing several deliveries of milk in glass bottles, observed that in practically all cases the barny flavors were partially or completely eliminated by pasteurization, but some feed flavors remained in the processed product. When the milk was heated to 142° F. (61.1° C.) and held for 90 minutes a cooked flavor, which was not apparent at the end of the 30 and 60 minute exposure, was apparent.

Marquardt and Dahlberg (1) found that pasteurization at 143.5° F. (61.9° C.) for 30 minutes not only diminished the intensity of feed flavor but blended the flavors of raw milk to give less variety in the pasteurized product. Usually they found it possible to select raw milk from pasteurized milk by the more pronounced feed flavor in the former.

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Trout and Taylor (4) found that holder pasteurization at 143° F. (61.6° C.) for 30 minutes changed the beet top flavor so that it could not be recognized as such, but did not improve the flavor of the milk to any appreciable extent.

Trout (5) reported finding feed flavors in 23.4 per cent of the samples of raw milk after one day of storage. On the other hand, he did not recognize any feed flavors in the pasteurized samples examined, although a wide variety of other flavors was noted.

Quinn and Burgwald (2) concluded that the high-temperature short-time pasteurization imparted less "cooked" flavor to the milk than did the holder method, but made no comment relative to the elimination of the feed flavors from the milk.

#### EXPERIMENTAL

The samples of milk used in this study were secured weekly, from January to June, 1939, inclusive, from ten producers who delivered milk to the College Creamery. Milk from these ten producers were used throughout the study.

Each of the ten weekly samples, divided into four lots, was processed as follows: Lot I, serving as a control, was stored at 40° F.; Lot II was holder pasteurized at 143° F. for 30 minutes in a tightly capped pint milk bottle so as to furnish no aeration during pasteurization and cooling; Lot III was similarly processed, but loosely capped in order to obtain aeration during processing; and Lot IV was pasteurized at 160° F. for 15 seconds by passing the sample through Pyrex glass 7 mm. tubing submerged in hot water and ice baths for appropriate heating and cooling. All samples were stored at 40° F.

After storage for 24 hours part of each sample, for organoleptic examination, was poured into a separate 100 ml. glass beaker and numbered on the bottom according to the key numbers of the samples. The 40 beakers of milk, consisting of raw, of pasteurized unaerated, of pasteurized aerated, and of flash pasteurized samples, were shuffled so that the judge had no knowledge of the sample being examined. After the flavor score and criticism of each sample were recorded the number on the bottom of the beaker was noted and recorded. Immediately following the completion of scoring of the 40 samples, they were reshuffled, rescored and the findings recorded as before, the record being kept on a second paper so the judge had no knowledge of the first score and criticism of the samples. On the third day of storage the samples were again scored and rescored exactly in the same manner as on the first day. Two experienced judges did the scoring throughout. Each judge's score was considered as an observation.

## RESULTS

*Flavors in the raw milk.* A critical study of the samples showed that on the first day of storage 43.3 per cent of the samples were free of flavor criticisms. Of the off-flavors noted, averaging 56.7 per cent, feed flavors predominated with 29.6 per cent; high acid flavors were next with 7.8 per cent; and flat flavors were third with 6.8 per cent. Ten other off flavors, present in a small percentage of the samples, were noted. The distribution of the observations on the samples of raw milk from the ten producers weekly over a six-month period according to flavor is presented in table 1.

TABLE 1

*Distribution of observations according to flavor in milk obtained weekly over a six-month period when pasteurized by various processes and when examined after the first and third days of storage at 40° F.*

Flavor	Distribution of observations when the milk was							
	Raw		Holder pasteurized, 143° F.—30 min.				Flash pasteurized, 160° F.—15 sec.	
			Un aerated		Aerated			
	1st day	3rd day	1st day	3rd day	1st day	3rd day	1st day	3rd day
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
No criticism	43.3	26.1	28.4	20.9	27.1	21.6	47.1	33.4
Bitter	0.2	0.0	—	0.2	—	0.2	—	0.2
Cooked	—	0.3	3.3	2.7	5.7	3.4	0.2	1.0
Cowy	—	2.1	0.2	0.7	0.8	1.8	0.7	1.3
Feed	29.6	21.5	19.6	12.5	16.8	10.2	19.9	15.6
Flat	—	6.8	5.4	5.0	3.8	6.2	6.7	5.7
Heated	—	2.4	3.6	34.0	30.3	29.2	25.7	13.5
High acid	—	7.8	21.4	2.2	2.3	2.2	1.3	2.1
Metallic	—	0.3	1.0	0.2	0.8	0.2	1.3	0.2
Off, unidentified	—	1.0	1.8	1.3	1.0	0.8	0.8	2.0
Old	—	1.8	6.0	1.3	4.8	3.7	6.7	9.4
Oxidized	—	1.5	4.4	2.6	18.4	4.0	18.6	2.4
Rancid	—	0.2	3.4	—	0.0	—	0.2	0.6
Salty	—	2.8	3.3	1.2	0.8	1.8	1.5	2.8
Unclean	—	0.3	1.6	0.3	0.5	0.3	1.2	0.6
No. observations	616	613	603	598	595	596	613	614

A study of these same samples after three days' storage at 40° F. showed marked decreases in the number of samples having no flavor criticism and of those having feed criticism. On the other hand, an increase was noted in the number of samples showing off flavors, the major increases being in the number of samples showing high acid, oxidized, and old flavors.

The percentage distribution of the observations on the samples showing specific scores is given in table 2. Here it will be noted that 43.3 per cent of the samples on the first day merited a flavor score of 23. However, by the third day of storage, the number was reduced to 26.1 per cent of the

TABLE 2

*Distribution of observations according to flavor scores in milk obtained weekly over a six-months period when pasteurized by various processes and when examined after the first and third days of storage at 40° F.*

Flavor score	Distribution of observations when the milk was							
	Raw		Holder pasteurized, 143 F.—30 min.				Flash pasteurized, 160 F.—15 sec.	
			Unaerated		Aerated			
	1st day	3rd day	1st day	3rd day	1st day	3rd day	1st day	3rd day
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
23 .....	43.3	26.1	28.4	20.9	27.1	21.6	47.1	33.4
22 .....	22.7	17.3	41.8	37.1	36.1	31.7	29.5	22.6
21 .....	20.6	24.6	23.4	22.7	27.7	22.0	17.5	24.8
20 .....	5.2	9.6	4.0	9.0	5.7	9.2	3.4	11.1
19 .....	3.0	4.4	1.3	5.8	1.7	9.6	1.1	5.4
18 .....	4.9	15.2	1.2	4.3	1.7	5.4	1.3	2.4
17 .....	0.2	1.0	.....	0.0	.....	0.2	.....	.....
16 .....	.....	0.6	.....	.....	.....	0.3	.....	0.3
15 .....	.....	1.1	.....	.....	.....	.....	.....	.....
No. observations .....	616	613	603	598	595	596	613	614
Mean .....	21.83	20.92	21.88	21.45	21.76	21.28	22.14	21.59
Standard deviation ..	± 1.35	± 1.41	± 1.07	± 1.34	± 1.10	± 1.50	± 1.08	± 1.30

samples. The mean flavor score on the first day was  $21.83 \pm 1.35$ , whereas, on the third day it was  $20.92 \pm 1.41$ , an average decrease of 0.91 points. This difference was found to be statistically significant.

The general quality of each producer's milk, by months, as indicated by the flavor score is shown in figures 1 and 2. As the summer season approached, there was a general lowering of the score due chiefly to the higher incidence of the feed flavors (figure 3).

*Flavors in the milk that was holder pasteurized without aeration.* A critical study of the samples of milk which were holder pasteurized without aeration showed that 28.4 per cent of the samples had no criticism on the first day of storage (table 1). Of the off flavors noted in 71.6 per cent of the samples, heated flavors predominated with 34.0 per cent, feed flavors were next with 19.6 per cent; and flat flavors were third with 5.0 per cent. Nine other off flavors, present in a small percentage of the samples, were noted. Pasteurization without aeration apparently was instrumental in lowering the frequency of feed flavors from 29.6 per cent in the raw to 19.6 per cent as judged on the first day of storage.

After three days storage there was a decrease in the number of samples having no criticism, or having feed, heated, flat, cooked and off flavors, and an increase in the frequency of old and oxidized flavors. The distribution

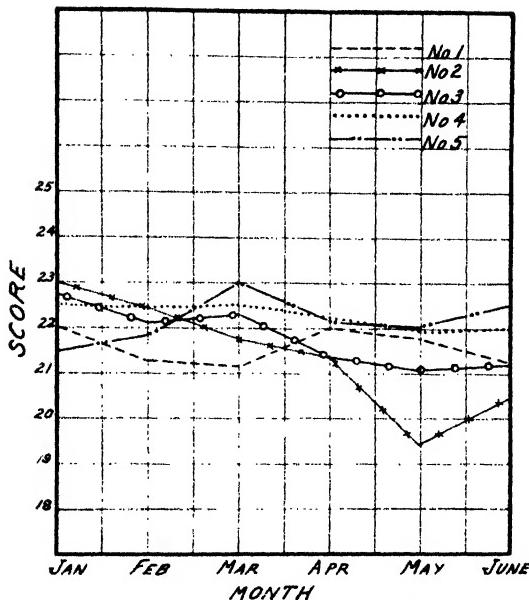


FIG. 1. The flavor score of herd milk from producers 1 to 5 inclusive over a six-months period.

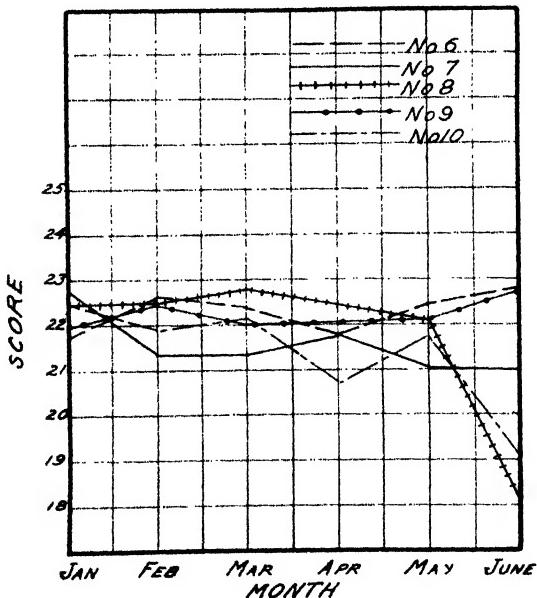


FIG. 2. The flavor score of herd milk from producers 6 to 12 over a six-months period.

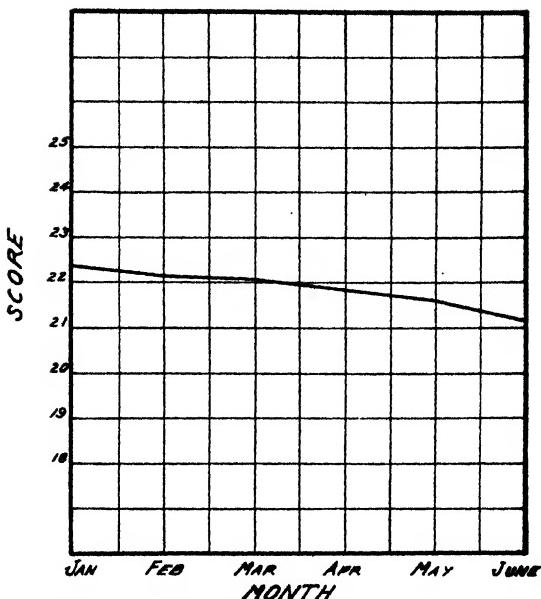


FIG. 3. Mean flavor score of mixed milk from ten producers over a six-months period.

of the samples having other flavors after three days storage remained practically the same as that noted on the first day of storage.

Low temperature holder pasteurization without aeration would seem to be partially effective in eliminating or reducing the intensity of such flavors as feed, high acid, cowy, flat, and rancid. On the other hand such pasteurization resulted in an increase in the frequency of cooked, heated, old, and oxidized flavors.

The mean flavor score of such processed milk on the first day of storage was  $21.88 \pm 1.07$ , whereas, on the third day the mean flavor score was  $21.45 \pm 1.34$  (table 2), a decrease in score of  $0.43 \pm 0.07$  which was statistically significant. The mean flavor score of the pasteurized milk samples, first and third day scorings combined, was found to be significantly higher than that of the combined first and third day scores of the raw milk samples. Likewise significant was the increase in score noted in the pasteurized milk over the raw milk after three days storage, but the increase on the first day was not significant.

*Flavors in the milk that was holder pasteurized with aeration.* The results secured by holder pasteurizing with aeration were similar in many respects to those secured in holder pasteurization without aeration (table 1). However, some variations were noted. For example, there was a further decrease in the number of samples showing feed flavors. Likewise, a lower

frequency of the heated flavor was noted. On the other hand, higher percentages of samples showing cooked, flat, and old flavors were observed. However, little difference was found between the unaerated and the aerated samples in the development of the oxidized flavor.

The mean flavor scores on the first and third days of storage were  $21.76 \pm 1.10$  and  $21.28 \pm 1.50$  respectively (table 2), a decrease in the mean flavor score of  $0.48 \pm 0.08$  point after a three-day storage period which was statistically significant. The mean score of the pasteurized aerated samples, first and third day score combined, was slightly higher than that of the raw milk scores combined, the significance of which represented a borderline case. The mean score of the combined first and third day scores of the pasteurized unaerated samples were significantly lower than the mean score of the pasteurized aerated samples. However, the lower scores for the respective days noted in the aerated versus the unacarated samples were not significant in either case.

*Flavors in the milk flash pasteurized.* On the first day of storage, 47.1 per cent of the samples flash pasteurized were free of flavor criticism. This percentage is 3.8 per cent higher than that observed in the control samples; 18.7 per cent higher than in the unaerated holder pasteurized samples, and 20.0 per cent higher than in the aerated holder pasteurized samples.

Of the off-flavors noted in 52.9 per cent of the samples, feed flavors persisted to the extent of 19.9 per cent, which was approximately the same as that noted in the unaerated holder pasteurized samples, but was 3.1 per cent greater than that noted in the aerated holder pasteurized samples. Heated flavors were present in 13.5 per cent of the samples, which is very significantly less than the 34.0 and 29.2 per cent noted in the two respective processes of the holder pasteurized milk. Cooked flavors, noted in a small percentage of the holder pasteurized samples were practically absent in the flash pasteurized samples.

A study of these same samples after storage, showed as, in the other milks, but to a lesser degree, an increase in the frequency of off flavors, 66.6 per cent of the flashed samples being so criticized as compared to 73.9 per cent in the raw, to 79.1 per cent in the unaerated holder pasteurized and to 78.4 per cent in the aerated holder pasteurized samples. Of the flavor criticisms, feed predominated with 15.6 per cent, oxidized with 13.5 per cent, heated third with 12.0 per cent, old fourth with 9.4 per cent, and flat with 5.7 per cent. Nine other off flavors were noted, but these occurred in relatively small percentages of the samples.

The mean flavor scores of the flash pasteurized samples were  $22.14 \pm 1.08$  and  $21.59 \pm 1.30$  on the first and third days of storage, respectively (table 2), the decrease of  $0.55 \pm 0.07$  point being significant.

The mean of the scores on the first day of storage of the flash pasteurized samples was significantly higher than the mean of the raw or holder pas-

teurized samples at similar storage. However, no significant difference was noted between the mean of the flavor scores of the pasteurized unaerated samples and that of the flash pasteurized milk when stored three days, although the difference between the mean scores of the pasteurized aerated and flash pasteurized samples under similar storage was significant. The mean score of any group of pasteurized milk after storage for three days was significantly higher than that of the raw milk samples similarly stored.

Combining the first and third day scores of each group, the means of the flash pasteurized samples were found to be significantly higher than those of the raw, of the unaerated pasteurized, or of the aerated pasteurized milk.

*Flavors tending to disappear or to develop as a result of pasteurization.* The data obtained indicate that two flavors, feed and high acid, tend to disappear markedly with pasteurization. Other flavors decreasing in intensity noted also were cowy, rancid, salty and unclean.

On the other hand the data show that pasteurization favors the appearance of such flavors as heated, cooked and oxidized. Other flavor tending to increase in frequency in the pasteurized samples was old, which is undoubtedly a degree of intensity of the oxidized flavor.

The flat flavors seemed to be current both with raw and with pasteurized



FIG. 4. The distribution of observations as to feed flavor in raw milk over a six-months period and the distribution as to oxidized flavor when the milk was holder pasteurized without aeration, illustrating the relationship noted between the two flavors.

milk, inclining perhaps to be slightly less prevalent in the unaerated holder pasteurized samples.

*The development of the oxidized flavor in the milk during the period of the study.* As previously shown, the oxidized flavor was noted in many of the samples of milk studied. Presented graphically in figure 4 is the percentage distribution of observations showing feed and oxidized flavors. As the frequency of feed flavor increased there was a marked decrease in the frequency of the oxidized flavor. A check on the management of the dairy herds from which the milk in this study was obtained showed that by the third week of April the majority of the producers had turned the cows to pasture, the grass contributing not only the causative agent of the feed flavor but apparently reducing substances inhibiting oxidation of the fatty constituents as well.

This observation on the decreased incidence of oxidized flavors in late spring or early summer is common to general commercial experience. The marked increase of feed flavors which occurred May first was due to the prevalence of grass flavors rather than silage feed flavor.

*Reliability of flavor judgments.* Two experienced judges scored all the samples "blind" and, after reshuffling the samples, rescored them, not knowing the previous score or criticism at the time of the second judgment,

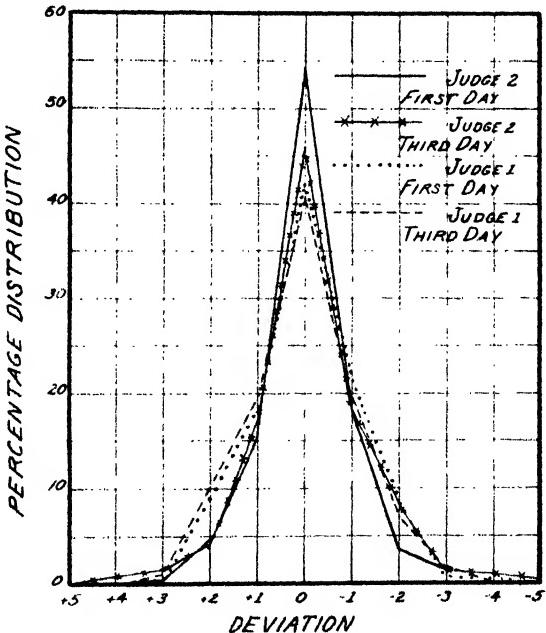


FIG. 5. Distribution curves of the deviation between the first and the second scores of each judge.

in an effort to determine first, the reliability of a single flavor judgment, and second, the closeness of scoring of the two judges. The samples were scored and rescored after the first and again after the third day of storage.

When the milk was scored after one day of storage at 40° F., Judge I rescored 42.0 per cent of the samples identically with the first score (figure 5). He deviated from the first scores on rescored by  $\pm 1$  point in 39.4 per cent of the 596 samples involved; by  $\pm 2$  points in 16.9 per cent of the samples; and by  $\pm 3$  or more points, the remaining 1.7 per cent. The tendency of this judge was to be more critical and to underscore the samples on second scoring. However, it must be borne in mind that the temperature of the milk rose between first and second judgments; hence, some off flavors might or might not have been detected on the second testing.

After the samples of milk had been in storage for three days they were again scored. The percentage of samples which were rescored identically with the first score was 40.8; those with a deviation of  $\pm 1$  were 39.4 per cent; those with a deviation of  $\pm 2$  were 17.5 per cent; and those deviating by  $\pm 3$  or more points were 2.3 per cent. Judge I had a tendency to rescore the samples higher than the first scores and with a greater range in score. The varying intensities of the flavors which predominated in the milk after three days of storage were such that made accurate rescored

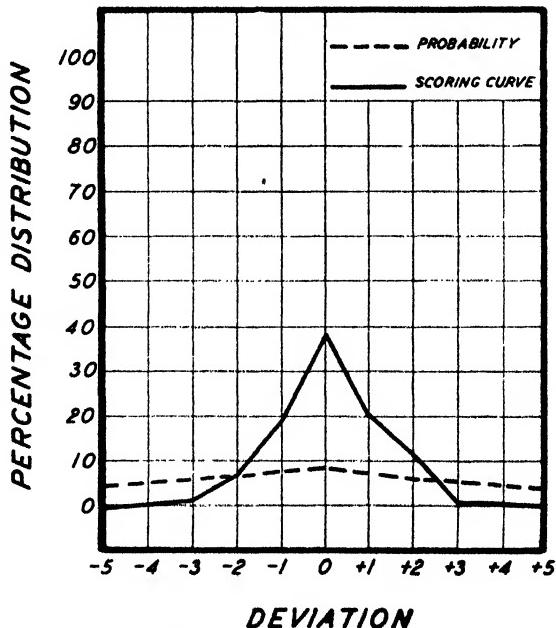


FIG. 6. Distribution curve of the deviation between the judges' scores on all samples of milk as compared to the distribution of probable deviation by random judgment.

difficult. However, little difference was noted in the judges scoring ability in rescored the fresh or the stored samples.

Judge II rescored 54.3 per cent of the samples of the day-old milk identically with the first score (figure 6). He deviated from his first score on rescored by a  $\pm 1$  point in 33.9 per cent of the 771 samples involved; by  $\pm 2$  points in 8.0 per cent of the samples; and by  $\pm 3$  or more points in 3.7 per cent of the samples. The tendency of this judge also was to be more critical and to underscore the day-old samples on rescored.

After the milk was stored three days they were again scored and rescored as before. The per cent of samples which were rescored identically with the first score was 46.8; those with a deviation of  $\pm 1$  point was 35.7; those with a deviation of  $\pm 2$  was 12.2 per cent and by  $\pm 3$  or more points in 5.3 per cent of the samples. Judge II had a tendency to rescore the samples higher and with a still greater range of score than Judge I.

The actual deviation curve between the first scorings of all the samples by the two judges is compared with the probability curve in figure 6. From this the conclusion may be drawn that some factor exists which tends to make the deviation between the judges' scores less than the sampling differences. The possibilities of like agreement by random sampling are so remote that there must be something specific, flavor, in milk which causes the judges not only to recognize its presence but to agree upon its intensity as well.

#### SUMMARY

Samples of milk from each of ten producers were secured weekly over a six-month period, divided into lots, were holder pasteurized with and without aeration at 143° F. for 30 minutes and flash pasteurized at 160° F. for 15 seconds. The samples were scored and rescored "blind" at the first and third days of storage by two judges working independently.

The predominating off flavor in the day-old, raw control samples was feed, the percentage frequency of which was materially decreased by pasteurization. The predominating off flavor of unaerated and aerated holder pasteurized day-old milk was heated. Less heated flavors were noted in the flash pasteurized samples than in the holder pasteurized samples. Flash pasteurized milk showed not only a higher percentage of observations of excellent flavor milk, score of 23, than were noted in the raw control or in the holder pasteurized samples but greater stability of excellent flavor upon storage as well.

No significant difference was found between the mean scores of milk holder pasteurized with aeration or similar pasteurization without aeration and that of the raw samples at the first day of storage. However, the increase in score of the pasteurized samples over the raw samples at three days of storage was significant.

Storing the milk for three days at 40° F. resulted in a significant decrease in the score over that noted at one day of storage. Raw milk scores decreased a mean of 0.91 points as a result of storage, whereas, the decrease in score of the pasteurized milk ranged from 0.43 to 0.55 points.

The pasteurization processes increased the frequency of heated, oxidized, cooked, and old flavors and decreased the frequency of feed, high acid, flat, salty, cowy, rancid, unclean, and off but unidentified flavors.

Storage of the milk at 40° F. for three days increased the frequency of high acid, old, oxidized, unclean, and rancid flavors in the raw samples and the oxidized and old flavors in the pasteurized samples, whereas, similar storage decreased the percentage incidence of feed, cowy, flat, heated, cooked, and off but unidentified flavors.

The mean score of the raw milk of all patrons decreased steadily from January through June. A gradual increase in the incidence of feed flavors was found in the raw samples from January to June. During the same period of time there was noted a rather constant frequency of oxidized flavors in the samples of the pasteurized milk, until May when the per cent frequency decreased markedly. As the frequency of feed flavors increased a very similar decrease in the occurrence of the oxidized flavors was noted.

The two judges varied slightly in rescoring ability, the one judge rescoring 42.0 per cent of the samples identically with the first score, whereas, the other judge rescored 54.3 per cent of the samples identically with the first score. Both judges, working independently, scored 39 per cent of all the samples with the same score.

#### ACKNOWLEDGMENT

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## THE EFFECT OF HOLDER AND FLASH PASTEURIZATION ON SOME FLAVORS OF MILK. II. THE EFFECT ON CORN AND ALFALFA SILAGE FLAVORS<sup>1</sup>

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Much feed-flavored milk is delivered daily to milk plants despite the availability of information on feeding management which, if carried out, would prevent the occurrence of such off flavors in the milk. General observation points to the fact that these off flavors are not confined solely to a specific season, although their frequency of occurrence may be greater at some seasons than at others. According to Babcock (1) feed flavors and odors are most frequently caused by succulent feeds. Undoubtedly the winter succulent, silage, plays an important role in the feed flavors of that season. Gamble and Kelly (2) showed that silage flavors were largely imparted to the milk through the body of the cow and were discernible in the milk from cows fed silage one hour before milking. They observed that legume silage fed in equal quantities as corn silage had the more detrimental effect on the flavor of the milk. These silage flavors were found to be partially removable by prompt aeration of the warm milk. They concluded that condensed milk made from silage-tainted milk had a less perceptible silage flavor and odor than the milk from which it was made. Roadhouse and Henderson (4) have recently shown that the feeding of ten pounds of corn silage one hour before milking produced a distinct feed flavor in the milk which was considered undesirable for market milk.

In view of the fact that these feed flavors are often current to milk during the winter season and appear subject to elimination by proper aeration, further studies seemed desirable to determine if such milk pasteurized by the various methods would be acceptable for bottling purposes.

### EXPERIMENTAL

The milk studied in these experiments was obtained from a selected healthy Holstein cow that was about two months in lactation at the beginning of the experiment and giving a good flow of milk. Exactly one hour before milking she was given alfalfa silage or corn silage, as the experiment dictated, in amounts varying from one pound to thirty-five pounds. The

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intensity of feed flavor was given a numerical rating obtained by dividing the pounds of silage fed by the pounds of milk produced. The milk was machine drawn into an aluminum container from which it was poured into a glass bottle which was placed in circulating water for cooling.

The corn silage milk was divided into six lots and treated as follows: Lot I served as a control; Lot II was pasteurized at 143° F. for 30 minutes without aeration; Lot III was similarly heat treated but was aerated by stirring during processing; Lot IV was holder pasteurized also but aerated by passing air up through the milk during the 30 minute holding period; and Lot V was holder pasteurized but subjected to a partial vacuum during processing; and Lot VI was flash pasteurized at 160° F. for 15 seconds and cooled without aeration. A seventh sample, designated as "trap" milk was secured by conducting the exhaust air from Lot IV through a similar volume of cold normal flavor milk, a sample of which was obtained as a control.

The alfalfa silage milk was divided into five lots of which one served as a control. The remaining four lots were holder pasteurized, unaerated and aerated by gentle agitation, by bubbling air through the milk, and by vacuum.

All samples were pasteurized in glass using laboratory equipment. The samples were cooled promptly and stored 24 hours at 40° F. after which they were scored "blind" by two judges. The samples were again scored at three day storage.

#### RESULTS

*The relationship between the quantity of corn silage fed and the feed flavor of the resulting milk.* The usual recommendation that a cow may be fed a certain amount of feed one hour before milking without imparting a feed flavor to the milk would appear to be largely dependent upon uniformity of production of milk per cow. As the milk production varies, the volume of flavor in the milk from a given quantity of feed would seem to vary also. Hence, it appears more logical to calculate the pounds of feed fed at a given time per pound of milk produced, in order to ascertain the relationship between the feeding of high flavor feeds and the feed flavor of the resulting milk. Several times during the experiment this assumption was checked by feeding a given quantity of silage to each of a group of individual cows varying in their level of milk production. The intensity of the flavor of the milk varied with the strongest flavor in the milk from the cow producing the least amount of milk. Accordingly, the intensity of the corn silage flavor in the raw control samples in these studies was expressed in terms of the number of pounds of silage fed one hour before milking per pound of milk produced.

Feeding trials showed that the cow could be fed up to 0.67 of a pound of corn silage per pound of milk produced one hour prior to milking without

imparting a feed flavor to the milk. On the other hand, the feeding of approximately 2.00 or more pounds of corn silage under the same conditions per pound of milk produced resulted in a strong feed flavor milk which scored approximately 18.0 to 19.0 on flavor (Table 1).

TABLE 1  
*The relationship between the quantity of corn silage fed and the flavor score of the resulting milk*

Feeding level below 2 pounds per pound of milk produced		Feeding level above 2 pounds per pound of milk produced	
Pounds of silage per pound of milk	Flavor score of the milk	Pounds of silage per pound of milk	Flavor score of the milk
0.50	23.0	2.10	17.0
0.67	23.0	2.34	18.5
0.79	20.0	2.50	18.5
0.90	19.5	2.60	18.0
0.94	21.5	4.00	19.0
0.99	18.0	4.20	21.0
1.00	20.0	5.00	18.0
1.05	21.0	5.10	18.0
1.30	19.0	6.20	18.0
1.60	19.5		
1.80	19.5		

Higher levels of feeding above a certain point did not seem to have a further detrimental effect on the flavor, probably because of the limited capacity of the cow to consume a given quantity of feed within a given period.

Dilution of several samples of silage flavor milk by normal flavor milk reduced the intensity of the feed flavor to a point where the silage flavor could not be detected. This effect would seem to have practical significance in the grading of milk where but a small percentage of the cans of milk had silage flavor.

*The effect of pasteurization on the flavor of corn silage milk.* Pasteurization had various effects on the removal of the feed flavor resulting from the feeding of corn silage depending upon the type of pasteurization and its modification (Table 2).

Some inconsistencies were noted in the results of holder pasteurization without aeration. In general, this method improved the flavor but the feed flavor was yet distinct. Likewise, holder pasteurization with gentle agitation by stirring or by forcing a current of air through the milk during the holding periods increased the flavor score materially but did not render it free of the distinct feed flavor. However, holder pasteurization under partial vacuum resulted in the removal of practically all the feed flavor. Milk originally scoring around 19 on flavor, when processed by this method usually scored 22 or above.

TABLE 2

*The effect of various methods of pasteurization on the score of corn silage flavor milk after the first day of storage at 40° F.*

Intensity of silage flavor (lbs. silage/ lbs. milk)	Flavor score when the sample was					
	Raw	Holder pasteurized, 143° F.-30 min.			Flash pasteurized, 160° F.- 15 sec.	
		Unaerated	Gentle agitation	Bubbling air		
4.00	19.0	.....	20.5	19.0	18.5	.....
2.50	18.5	21.0	21.5	22.0	22.0	21.5
1.80	19.5	22.0*	21.5	21.5	22.5	22.5
1.60	19.5	19.5	21.5	21.2	22.0	21.0

\* Scores italicized indicate that such milk would likely not be criticized as to taste by the average consumer.

The superiority of partial vacuum holder pasteurization of feed-flavored milk was noted particularly after the milk had been stored for three days. During this period oxidized flavors were prone to develop in the samples holder pasteurized without vacuum, whereas the vacuum pasteurized samples retained the good flavor. This is in agreement with the work of Hand, Guthrie, and Sharp (3) who showed that vacuum cooled milk was less susceptible to oxidative changes.

Flash pasteurization seemed to be on a par with ordinary holder pasteurization in the removal of strong corn silage feed flavor from the milk.

That the corn silage flavor was susceptible to removal by aeration and pasteurization was demonstrated by the fact that gases above the silage milk during processing when conducted through milk, free of feed flavor, imparted a feed flavor to the sample thus treated.

*The effect of pasteurization on the flavor of alfalfa silage milk.* Samples of milk with various intensities of alfalfa silage flavor were holder processed as in the previous experiment. The intensity of the flavor in the original raw milk was again expressed in terms of the pounds of alfalfa silage fed one hour before milking per pound of milk produced.

The alfalfa silage flavor was also volatile and could be transferred, in part, by drawing air through the feed flavor sample into one of excellent flavor, to the extent that a feed flavor could be imparted to the latter.

As in the experiment with corn silage flavor milk, partial vacuum holder pasteurization was again superior to the other modifications of holder pasteurization in yielding a product which was comparatively free from feed flavor and which would merit a score of 22.0 or above on flavor (Table 3). Aeration by bubbling air through the milk during the holding period was partially effective in reducing the alfalfa silage flavor, but practically no difference was noted between the mean flavor scores of the raw and of the pasteurized unaerated samples or of the pasteurized samples aerated by gentle agitation during the holding period.

TABLE 3

*The effect of various methods of pasteurization on the score of alfalfa silage flavor milk after the first day of storage at 40° F.*

Intensity of silage flavor (lbs. silage/lbs. milk)	Flavor score when the sample was				
	Raw	Holder pasteurized, 143° F.-30 min.			Aerated by
		Unaerated	Gentle agitation	Bubbling air	
2.0	20.0	19.0	20.0	22.0	22.5
1.6	21.0	<i>22.0*</i>	21.0		
1.2	21.5	21.5	21.0	19.0	21.0
0.73	21.5	21.0	20.0	21.5	22.0
0.42	18.0	21.5	21.5	<i>22.5</i>	<i>22.5</i>
0.40	20.5	20.0	19.0	<i>22.0</i>	<i>22.0</i>
0.22	<i>23.0</i>	21.5	21.5	<i>22.0</i>	<i>22.0</i>
0.18	21.5	<i>22.5</i>	<i>22.0</i>	<i>23.0</i>	<i>23.5</i>
0.08	<i>23.0</i>	<i>22.0</i>	<i>23.0</i>	<i>23.0</i>	<i>23.0</i>
Mean .....	21.1	21.2	21.0	21.8	22.1

\* Scores italicized indicate that such milk would likely not be criticized as to taste by the average consumer.

A marked difference was noted in the flavors of the milk resulting from the various methods of pasteurization when the milk was stored for three days at 40° F., particularly in respect to pasteurizing under partial vacuum. The partial vacuum pasteurized milk tended to retain its more excellent flavor, whereas different stages of oxidation were noted in many of the samples not vacuum pasteurized.

#### SUMMARY

When cows were fed a given quantity of silage the feed flavor was more intense in that milk from the cows of least production. Feed flavors were noted in the milk when 0.79 pounds of corn silage or 0.40 pounds of alfalfa silage per pound of milk produced were fed to the cows one hour before milking.

Alfalfa and corn silage flavors in milk were lessened in intensity by pasteurization. However, strong silage flavors were not entirely eliminated by the processes employed.

Vacuum holder pasteurization and forced aeration holder pasteurization were superior to unaerated or aerated holder pasteurization in removing corn and alfalfa silage flavors from milk. These processes were superior also to flash pasteurization in removing corn silage flavor from milk. Unaerated and aerated holder pasteurization resulted in a greater frequency of oxidized flavors in the stored milk than did vacuum pasteurization.

From these studies it appears that a small quantity of silage flavor milk may not necessarily taint the flavor of a large batch of processed milk. Suf-

ficient excellent flavor milk may be added to the silage milk to reduce the flavor intensity to the extent that pasteurization will remove it entirely.

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PHYSICAL AND CHEMICAL PROPERTIES OF THE FAT GLOBULE  
ADSORPTION "MEMBRANE." II. NATURE AND ORIGIN  
OF SURFACE ACTIVE MATERIALS INVOLVED IN  
CURD TENSION REDUCTION AND PREVEN-  
TION OF RENNET CLOT OF COW'S MILK  
BY "MEMBRANES" FROM NATURAL  
AND SYNTHETIC CREAMS\*

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*Foreword:* This paper is one of a series giving the results of studies carried out more or less simultaneously in the two laboratories designated in the authorship of this paper. These studies have proceeded with a free exchange of results and frequently of detailed data. The second part of the present paper is a portion of the joint study and contains data obtained in the California laboratory.

INTRODUCTION

In an earlier paper we (1) showed that reduced or very low curd tensions are exhibited by artificial "buttermilks" produced by churning synthetic creams whose fat globule adsorption "membranes" are derived from (a) washed sweet cream, (b) sweet rennet whey and (c) sols of rennet whey powder. Skim milk containing added natural fat globule "membrane" complex also exhibited reduced curd tension. On the other hand, normal curd tensions were exhibited by "buttermilks" when skim milk was employed as emulsifying agent or when a lecithin-cephalin mixture (80-85 per cent lecithin) was added to skim milk. The conclusion drawn at that time that the fat globule "membrane" itself, particularly its protein component, causes the reduced curd tension of natural buttermilk is supported by our (2) later experiments in which we employed fat globule adsorption "membranes" derived from sols of skim milk powder, calcium caseinate, gelatin, tissue fibrinogen (lecithoprotein) and whey powders (from both rennet and acid whey). When the adsorption "membrane" of the artificial creams was derived from whey powder, gelatin and calcium caseinate, the buttermilk exhibited very low curd tension. When gelatin and calcium caseinate furnished the "membrane," the addition of relatively large amounts of  $\text{CaCl}_2$  largely, if not completely, prevented the low curd tension

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of the buttermilk but there were indications that this was not due merely to supplying  $\text{Ca}^{++}$  needed for normal clotting.

The experiments to be reported in the present paper support the conclusion that two somewhat different types of phenomena were involved in our previous results. One type appears to involve a partial denaturation of fat globule "membrane" protein which prevents normal curd tension of the buttermilk, the intermediate steps not yet being clearly understood. In the other type natural esterase in raw milk plasma liberates fat acids which prevent the normal clotting of milk if certain conditions are provided. The application of these findings to natural sweet cream buttermilk will be considered in another paper.

#### EXPERIMENTAL

*Procedures and methods.* The general procedures were briefly as follows. Pure, melted butter fat was emulsified at 37° C. in a suitable quantity of the emulsifying agent to produce a synthetic cream containing 27–30 per cent fat. After dilution with skim milk to 3–4 per cent fat content, the "remade" whole milk gave, on centrifugal separation, "remade" skim milk and "remade" cream. The cream, on churning, produced "remade" buttermilk. Any cream which was subjected to washing was diluted with at least four volumes of distilled water in each washing. When it became necessary to concentrate any preparation obtained in this study the concentrate was effected by pervaporation.<sup>1</sup>

The methods for determination of curd tension, surface tension, N content, etc. were described in previous papers (1, 2). In the present study pH was determined in some cases by the hydrogen electrode and in others by the quinhydrone electrode. Any special procedures employed or modifications of the usual ones will be described in connection with their particular use.

1. *Evidence bearing on protein denaturation being involved in the low curd tension of buttermilk.*<sup>2</sup> It is recognized that the term protein denaturation has not yet been defined exactly but it seems to be generally recognized at present that it is the result of intramolecular change, involving the appearance of SH groups, causing a loss of one or more properties, especially that of "solubility." We have previously (1, 2) shown that the curd tension of "remade" buttermilks containing "membrane" proteins derived from whey or whey powder is much lower than when the buttermilks contain natural "membrane" protein. Since whey proteins are definitely

<sup>1</sup> Pervaporation is the term applied by Kober (J. Am. Chem. Soc. 39: 944, 1917) to the spontaneous evaporation of water from colloidal sols through a semipermeable membrane which encloses them. Our method of performing pervaporation is described in a previous paper (1).

<sup>2</sup> The experimental data presented in this section of the paper were obtained at the University of Minnesota and are taken from the thesis presented by N. P. Tarassuk for the Ph.D. degree, University of Minnesota, 1937.

heat coagulable whereas the isolated "membrane" protein shows little if any heat coagulability (3) the possibility is suggested that a reduction in curd tension of a buttermilk may be determined, in part at least, by the extent of denaturation of the "membrane" protein and its release into the buttermilk. It is now established that protein denaturation may be brought about by surface energy as well as by heat. It is certain that surface forces are involved in the formation of an emulsion and in the churning of cream to butter. Also it has been known since the early observation of Ascherson (4) that a denaturation of albumin occurs at an oil-albumin sol interface and that this is accentuated by shaking the mixture.

A discussion of the various prevailing theories regarding the structural and chemical changes occurring in protein denaturation by different forms of energy will not be attempted, but certain results observed in recent studies on surface coagulation of albumin will be cited since like results occurred in the curd tension studies of "remade" buttermilks involving natural whey and whey powder sols. It has been shown by Bull and Neurath (5) that the pH of egg albumin sols shifts during surface denaturation, the direction and extent of shift depending upon the initial pH of the sol. A decrease of 0.2-0.3 pH was found to occur at pH 6.6. In these experiments the rate as well as the extent of surface denaturation at pH 6.6 was relatively low even when other conditions (higher protein concentration and presence of electrolytes) were favorable. Also, they found that even under conditions of maximum coagulation not as much protein could be coagulated by shaking as by heating, although the noncoagulated portion remaining in the heated sample would still show surface denaturation.<sup>3</sup>

If one accepts Neurath's (6) theory that surface denaturation of protein represents an unfolding of the molecule, decreased surface tension should be found to accompany the denaturation. Although this does not seem to have been studied systematically, indirect evidence for it is found in the experiments of Danielli (7) showing that interfacial tension is permanently decreased at an oil-water interface, in the presence of protein.

We admit at the outset that the experimental evidence which we have so far obtained is not as conclusive as we would desire in support of the hypothesis that denaturation of fat globule "membrane" protein may be involved in certain cases of reduced curd tension of buttermilk. The experimental fact that low curd tension is accompanied by lower pH and lower surface tension in the whey protein experiments already published (2) could be accepted as presumptive evidence of protein denaturation when viewed in the light of the foregoing discussion. Since the same effects are produced as the result of milk esterase activity, provided certain fat acids are liberated, as will be shown later in this paper, conclusive evidence for

<sup>3</sup> Protein which has undergone surface denaturation at pH above or below its isoelectric point will coagulate when the pH is brought to this point.

protein denaturation obviously must be sought under conditions which do not suggest esterase activity but where protein denaturation can occur and curd tension is affected.

(a) *Effects on curd tension of direct addition to milk of isolated fat globule "membranes."* If it be assumed that denatured fat globule "membrane" protein may reduce the curd tension of buttermilk when released during the churning of cream, it seems reasonable to expect the same result if the "membrane," isolated from washed cream, is added directly to skim milk. Such a result should be most evident if the "membrane" protein is readily denatured, e.g., in the case of an artificial cream made by dispersing butter fat in a sol whose proteins are exclusively those of milk whey.

Experiments I, II and III, table 1, give the results of tests based on the above hypothesis. In each experiment the principal curd tensions given for comparison are those of (1) the untreated control skim milk, (2) the skim milk plus a "membrane" sol and (3) the skim milk plus water equal to the volume of "membrane" sol tested; the purpose of the last test being to determine the effect on curd tension attributable to the dilution of the skim milk with the "membrane" sol. In experiment II there was an additional comparison in which there was added to the control skim milk a sol of the whey powder having the same concentration of fat and solids-not-fat as in one of the "membrane" sols tested in this experiment. It was thought that this would give additional evidence regarding any change affecting curd tension, which might have occurred in the whey proteins during the preparation and churning of the emulsion.

In experiment III, table 1, an attempt was made to test the possibility that the denatured portion of the "membrane" protein is not confined exclusively to that portion of the "membrane" which remains on the surface of the fat globules after they are washed but is, in part, removed by the washing operation. It is already established (8) that the first washing of cream removes most of the plasma proteins and undoubtedly most of the loosely adsorbed "membrane" also. Regardless of how the denatured portion of "membrane" protein distributes itself in such an experiment, it is safe to say that the first washing plus the free buttermilk from the cream thus washed will contain all of the plasma proteins in the original unwashed cream as well as most of the denatured protein. Some of the "membrane" protein remains in the butter; this portion was not included in our study.

The "membrane" sols tested in experiment III were in one case a concentrate of the first washing of a "remade" whey powder cream and in the other case a mixture of this concentrate and some of the "membrane" from the washed cream. The concentrate of the first washing was added to skim milk when its effect on curd tension was tested alone. When the mixture was tested no plasma proteins were involved except those in the first washing concentrate, the curd tension being determined on a sol made up of nine

parts of this concentrate containing 7.85 per cent s.n.f. and one part of buttermilk concentrate from the washed cream, the latter also containing 7.85 per cent s.n.f.

Since the procedure employed for preparing the several "membrane" sols employed in experiments I, II and III, table 1, differed somewhat, a brief description of each sol seems desirable.

TABLE 1  
*Curd tension reductions when artificial fat globule "membranes" are added directly to raw skim milk*

Sample No.	Description of sample	Curd tension	pH	Surface tension
<i>Experiment I</i>				
1	Raw skim (blank)	77	...	...
2	210 ml. raw skim + 16.9 ml. "membrane" sol No. 1	60	....	...
3	210 ml. raw skim + 16.9 ml. H <sub>2</sub> O	64	....	...
<i>Experiment II</i>				
4	"Remeade" skim (blank)	52	...	...
5	90 ml. "remade" skim + 10 ml. "membrane" sol No. 2	27	....	...
6	90 ml. "remade" skim + 10 ml. "membrane" sol No. 3	35	....	...
7	90 ml. "remade" skim + 10 ml. H <sub>2</sub> O	41	....	...
8	90 ml. "remade" skim + 10 ml. whey powder sol*	40	....	...
<i>Experiment III</i>				
9	Raw skim (blank)	79	....	...
10	90 ml. raw skim + 10 ml. "membrane" sol No. 4†	60	....	...
11	90 ml. raw skim + 10 ml. H <sub>2</sub> O	69	....	...
12	90 ml. "membrane" sol No. 4 + 10 ml. "membrane" sol No. 5	0	....	...
<i>Experiment IV</i>				
13	Raw skim (blank)	70	...	50
14	Raw skim + 25% "membrane" sol No. 6	36	6.53	47
15	Raw skim + 25% H <sub>2</sub> O	37	6.61	50
16	Raw skim + 14.5% "membrane" sol No. 7	53	6.46	46
17	Raw skim + 14.5% H <sub>2</sub> O	53	6.62	50

\* This sol had the same concentration of fat and solids-not-fat as "membrane" sol No. 3.

† "Membrane" sol No. 4 had total solids = 11.85%, fat = 4.0%, pH = 6.54.

"Membrane" sol No. 1: A 30 per cent cream was prepared by emulsifying butter fat in nine per cent aqueous dispersion of spray dried rennet whey. The cream was held for 26 hours at 8° C., washed twice\* at 37° C.,

\* Chemical analyses of these washings and buttermilk obtained after churning showed 0.035% N in first washing, 0.018% N in the buttermilk and 0.003% N in the second washing.

aged for several hours at 8° C., churned, and the free "buttermilk" concentrated to 3.6 per cent of the original volume. This concentrate constitutes "membrane" sol No. 1.

"Membrane" sol No. 2: This was prepared in the same manner as sol No. 1 except that the synthetic whey powder cream was aged in ice water for four hours and the free "buttermilk" from the twice washed cream was concentrated to only 8.3 per cent of the original volume. The concentrated "buttermilk" constitutes "membrane" sol No. 2.

"Membrane" sol No. 3: A portion of the same 30 per cent cream used for sol No. 2, which had been aged in ice water, was diluted with five volumes of raw skim milk to produce a "remade" whole milk. This was reseparated to produce a "remade" cream. The cream was held overnight at 8° C., washed once at 37° C., churned, and the free "remade buttermilk" concentrated to 6.3 per cent of its original volume. This concentrate constitutes "membrane" sol No. 3. The "remade" skim milk obtained in the course of preparing this sol was employed as the control milk in experiment II.

"Membrane" sol No. 4: A 30 per cent cream in a nine per cent aqueous dispersion of the dried whey was diluted with five volumes of raw skim milk to produce a "remade" whole milk. This was held overnight at 8° C., washed once at 37° C. using four and one-half volumes of water. The first washing was concentrated until it contained 7.85 per cent s.n.f., this being the average s.n.f. content of free "remade" buttermilk from unwashed whey powder cream. This concentrate constitutes "membrane" sol No. 4.

"Membrane" sol No. 5: This preparation is a concentrated sol of the free "buttermilk" from the churned, "remade" washed cream whose first washing was used to produce "membrane" sol No. 4. It was concentrated until it contained the same s.n.f. as "membrane" sol No. 4.

(b) *Evidence that protein denaturation can occur in butter fat surfaces during emulsification and churning.* It was found in the course of our studies that excellent emulsions capable of being churned readily could be produced with butter fat and an aqueous sol of undenatured protein<sup>5</sup> remaining in rennet whey powder after extraction of all lipides by hot 75 per cent ethyl alcohol followed by ethyl ether. These emulsions furnished some evidence of further denaturation as follows:

1. Emulsification. When a concentrated sol of the protein was shaken mechanically in glass for 48 hours at 10°-12° C. both at its original pH (6.35) and at pH 4.85, there was distinct evidence of increased cloudiness

<sup>5</sup> The undenatured, water soluble protein was isolated by a modification of the method described by Rimpila and Palmer (8) for isolating fat globule "membrane" protein. We employed only three volumes of dioxan to one volume of dialyzed, lactose free, filtered protein solution for flocculating the protein, thus permitting the protein to redisperse in water if not subjected to drying. The isolated protein was found to contain only 10.31 per cent nitrogen. The significance of the low nitrogen, which makes it resemble the natural fat globule membrane protein, remains to be discovered.

only at the low pH. However, when the sol was shaken for 30 minutes at 37° C. in the presence of 20 volumes per cent butter fat, and the emulsion centrifuged at high speed, the "skim milk" layer showed evidence of surface denaturation when brought to pH 4.8, especially if the fat emulsion remaining in the lower layer was partially broken by adding one-third volume of ethyl ether.

2. Emulsification and churning. When creams made from butter fat and the protein sol were churned, the buttermilk showed distinct precipitation on acidification to pH 4.0-4.6. It would be expected that such buttermilks should cause a reduction in curd tension when added directly to raw skim milk but this was not found to be the case as shown in experiment IV, table 1. In one test the unaltered buttermilk was employed ("membrane" sol No. 6) and in the other it was first concentrated to 17 per cent of original volume ("membrane" sol No. 7).

#### DISCUSSION OF DENATURATION EXPERIMENTS

Experiments II and III furnish the strongest evidence for the possibility of a protein denaturation relationship to reduced curd tension. It should be emphasized that the "membranes" employed in these tests did not remain in contact with milk caseinate for long periods prior to the determination of curd tension so that there was not the opportunity for physico-chemical changes to occur gradually which would prevent the action of rennin. Instead, the "membranes" were added directly to the milk and the curd tension determined almost immediately.

The results show that the effects of "membrane" sols No. 2 and 3, especially No. 2, were clearly greater than obtained by dilution. There is evidence in the same direction, although not in itself convincing, for "membrane" sol No. 1. There is no possibility that lipolytic action could have been involved in the case of "membrane" sol No. 2 for it was not in contact with raw skim milk except at the time of the curd test. Raw skim could have contributed lipolytic enzymes to supply fat acids in the case of "membrane" sol No. 3 but it does not seem likely that it did because "membrane" sol No. 3 had less effect on curd tension than "membrane" sol No. 2. The effect of "membrane" sol No. 4, experiment III (from first washing of a "remade" whey powder cream) was also clearly greater than could be accounted for by dilution and, when combined with the buttermilk from this cream to form "membrane" sol No. 5, did not clot at all when rennet was added. Some of the latter effect admittedly could have been due to lipolysis occurring during the pervaporation of the "remade" buttermilk but it seems unlikely that this was the sole cause since "membrane" sol No. 5 was analogous to "membrane" sol No. 3 which did not completely prevent clot formation.

Special significance possibly should be attached to the curd tension reducing properties of "membrane" sol No. 4 for this represented the outer

"membrane" of the emulsion, removable by washing. The result suggests either that the outer portion of such an adsorption layer is more readily denatured by surface energy or that the portion which has become denatured is more readily detached. The latter view is more in keeping with Neurath's (6) theory of surface denaturation of protein molecules.

It is not possible to explain readily the failure of "membrane" sols No. 6 and 7 to reduce curd tension more than obtained by dilution alone. The stabilizing "membranes" from which these sols were derived were, of course, undenatured residues from the hot alcohol treated whey powder. Although evidence was obtained that they could be denatured further by surface energy, it is possible that not enough further denaturation occurred to affect curd tension. In support of this is the evidence that they had very little effect on either pH or surface tension of raw skim milk.

2. *The effect of esterase activity on curd tension.* The results to be described were the outgrowth of an effort to obtain further light on the question whether a "membrane" protein denaturation is an important factor in the low curd tension of buttermilk. In our previous studies (2) curd tension of buttermilk was either greatly reduced or clotting completely prevented when the membrane was composed of either denaturable proteins (whey proteins) or non-denaturable proteins (casein or gelatin). It seemed desirable to study the effects of employing a "membrane" which was neither protein nor phospholipide, if such could be obtained. We found that diglycol laurate, which is an excellent emulsifying agent, would serve the purpose. The results obtained opened up entirely new aspects of the problem of reduced curd tension and led to the establishment of some of the conditions under which the activity of natural lipolytic enzymes in milk may be responsible for the complete prevention of coagulation by rennet. We will present here only a few crucial experiments among a large number carried out on various aspects of the problem.

(a) *Study of diglycol laurate cream.* A synthetic cream was made by emulsifying 250 ml. fresh, filtered, melted butter fat with 600 ml. six per cent aqueous diglycol laurate suspension at 37° C., using the hand emulsifier, the mixture being put through four times. The emulsion was diluted immediately with 5,720 ml. fresh raw skim milk and the "remade" whole milk separated at once at 38°–40° C. Separation was normal. The remade cream appeared normal after standing overnight in a cold room. Churning at 55°–60° F. required 1.5 hrs., the gathering of the butter granules being slow. The total solids content of the original skim, the "remade" skim and the "remade" buttermilk was 8.5, 8.19 and 8.7 per cent respectively, the fat content of the corresponding products being 0.02, 0.49 and 0.90 per cent respectively. Data on the curd tension with rennet alone at 35° C., the pH and the surface tension are given in table 2, experiment I. All results are averages of at least two determinations.

TABLE 2

Curd tension at 35° C., pH and surface tension of original and "remade" milks from diglycol laurate cream and of skim milk after direct addition of the same fat acid ester

Sample No.	Description of sample	Curd tension		pH		Surface tension Fresh dynes/cm.
		Fresh	After aging	Fresh	After aging	
<i>Experiment I</i>						
1	Original skim milk	62	...	6.60	...	53.9
2	"Remade" whole	35	...	...	...	...
3	"Remade" skim	50	49*	6.58	...	45.5
4	"Remade" buttermilk	3	1*	6.20	...	41.3
5	"Remade" skim + 30% H <sub>2</sub> O	25	...	...	...	...
<i>Experiment II</i>						
		After 16 hrs.†	After 40 hrs.†	After 16 hrs.	After 40 hrs.	After 16 hrs.
1	Original skim milk	56	56	6.62	6.60	53.2
2	Skim agitated 30' at 23° C.	51	54	6.66	6.60	53.2
3	Skim + 0.5% diglycol laurate, stirred	41	14	6.45	6.35	35.7
4	Skim + 0.5% diglycol laurate, agitated like sample 2	0	0	6.34	6.20	39.2
5	Skim agitated 30' at 7°-10° C.	55	54	6.62	...	53.2
6	Skim + 0.5% diglycol laurate, agitated like sample 5	7	0	6.34	6.25	36.4

\* Aging was for 22 hours at 7°-10° C.

† Aging was at 7°-10° C.

(b) *Effect of direct addition of diglycol laurate to skim milk.* Diglycol laurate was emulsified into raw skim milk and the effect on curd tension with rennet alone at 35° C., pH and surface tension studied at intervals. The conditions of the experiment, which had suitable controls, as well as the results are given in table 2, experiment II. Agitation was carried out in a motor driven, four quart Dazey churn having a two blade paddle revolving at approximately 250 r.p.m. All results are averages of at least two determinations.

It is apparent that diglycol laurate cream shows the same phenomenon as synthetic protein creams and that like results are obtained simply by agitating of skim milk containing a dispersion of diglycol laurate. The first explanation which suggests itself is that diglycol laurate is adsorbed by the calcium caseinate, thereby preventing normal rennet action. But this would not of itself explain the simultaneous decline of both curd tension and pH on standing when the ester is added directly to the milk. These effects were repeatedly confirmed. In one of the confirmation experiments the further significant fact was brought out that the use of CaCl<sub>2</sub> in the curd test prevented the deleterious effect of the ester on the tension. For example, a

sample of raw skim milk, treated with diglycol laurate, showed a curd tension of 50 grams at 35° C. and a pH value of 6.60 when fresh and a curd tension of zero at 35° C. and a pH value of 6.15 after 40 hours aging at low temperature but the zero value was raised to 61 grams on addition of four volumes per cent of 5 per cent  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  solution before addition of the rennet. Analogous effects of  $\text{CaCl}_2$  were reported in our previous paper (2) when studying the curd tension of "remade" buttermilk from synthetic gelatin and calcium caseinate creams.

The correct explanation of clot prevention in these experiments was found in a study of the cause of the gradual decline in pH. This was found to be due to the liberation of lauric acid from the diglycol laurate ester. The experimental proof will be presented in detail in another paper but experiments I and II, table 3 show clearly that pasteurization of skim milk (30' @ 65° C.) completely prevents the adverse effect on the rennet clot which results when diglycol laurate is dispersed in raw skim milk.

TABLE 3

*Effect of previous pasteurization on the changes in curd tension, pH and surface tension in agitated, diglycol laurate treated skim milk*

Sample No.	Description of sample	Curd tension		pH		Surface tension dynes/cm. A
		A*	B*	A	B	
1	<i>Experiment I</i>					
	Past. (blank)	gms. 69 (21)	gms. 62 (44)	.....	6.64	52.5
2	Past. + 0.5% dg.l., † agitated	41	45	.....	6.68	33.6
3	<i>Experiment II</i>					
	Raw (blank)	64 (22)	68 (72)	6.69	6.63	.....
	Raw + 0.55% dg.l., agitated	0	0	6.39	6.22	.....
4	Past. + 0.55% dg.l., agitated	35	44	6.74	6.73	.....
5						

\* The figures in parentheses indicate the number of hours the sample was aged at low temperature before the datum was obtained.

† Dg. l. is abbreviation for diglycol laurate.

#### SUMMARY AND CONCLUSIONS

Experiments are described showing a reduction in curd tension when artificial fat globule "membrane" sols derived from spray dried whey were added directly to natural or "remade" skim milk. Since some of these sols exhibited evidence of protein denaturation on shaking, the experiments suggest that this may interfere, under some conditions, with normal clotting of milk by rennet.

Experiments are described showing that the normal rennet clot may be completely prevented by emulsifying a small amount of diglycol laurate into raw milk at room temperature, aging the emulsion in the cold and add-

ing rennet at 35° C. This phenomenon also occurs in "remade" buttermilks from "creams" whose butter fat globule "membrane" is diglycol laurate. A decrease in surface tension and pH accompanies the destruction of clotting ability. The explanation of this phenomenon is the liberation of lauric acid from the diglycol ester by natural milk enzyme. It does not occur if the milk is first pasteurized.

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# SPERM STIMULATION IN THE BULL THROUGH THE SUBCUTANEOUS ADMINISTRATION OF ASCORBIC ACID\*

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Ascorbic acid therapy for impotency in the male bovine has given rather startling results. The initiation of such treatment was based upon three facts obtained from rather widely separate observations. Phillips and Stare (1) in 1933 reported a very high concentration of ascorbic acid in the pituitary gland of cattle. Phillips *et al.* (2) reported preliminary evidence to show that a low blood plasma ascorbic acid was found in cattle fed on a restricted dietary regimen. Lardy and Phillips (3) found that bulls with low fertility showed less than 2 mg. of ascorbic acid per 100 cc. of fresh semen and in some cases only a trace. Good breeding bulls on the other hand produced semen containing from 3.0-8.0 mg. of ascorbic acid per 100 cc. of fresh semen. With these facts at hand ascorbic acid therapy was undertaken.

Bulls of low potency rating, slowness in breeding and general sexual indifference were sought. Such animals are difficult to obtain because poor breeding bulls are soon dispensed with. A number of such animals have been placed in our hands for treatment. The results obtained seem to warrant publication at this time.

In general, the laboratory data rest largely upon the ascorbic acid content of the blood plasma and semen, the longevity in storage in our yolk buffer pabulum, microscopic observations, and upon return to active breeding service. The ascorbic acid analyses were determined by the method of Mindlin *et al.* (4). Longevity records were obtained by microscopic observations at 37° C. made at 24 hour intervals. Samples thus observed were given ratings of 1 + to 4 + depending upon the type and vigor of the sperm movement. One plus represented poor motility, while 4 + indicated excellent quality semen. The end-point for longevity recording was the change from 1 + to less than 1 + or at the point when only a few sperm were moving.

In all cases herein reported ascorbic acid was injected subcutaneously at the rate of about 1 gram per 1000 pounds of live weight. Such injections

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were made twice weekly where possible. Some cases in the field were given 2 gram doses at five day intervals. Occasionally, it was impossible to give regular periodic treatments where travel and distance were involved. The results obtained indicate that the size of dose should be at least 1 gram per 1000 pounds of live weight. The dose has been increased to 1½ grams per 1000 pounds live weight with good results. Larger doses seem to be unnecessary.

#### RESULTS

The results from these studies have been gratifyingly satisfactory. Table 1 shows the progressive recovery of potency by bull No. 1220 under ascorbic acid treatment. It is to be regretted that blood samples were not obtained in this case. It is seen, however, that there was no ascorbic acid in the semen sample obtained prior to treatment. Ascorbic acid began to appear in the semen after 2 or 3 weeks. Thereafter, there was rapid recovery until at the end of 5 weeks the ascorbic acid content of the semen reached the normal

TABLE 1

*The effect of ascorbic acid therapy upon bull 1220,\* the first bull treated with ascorbic acid*

Date	Semen ascorbic acid	Longevity in yolk buffer	Total ascor- bic acid injected	Character of semen
7/28/39	mg. %	hr.	gm.	
7/31/39	0	0	0	Thin, watery, no motility
8/ 9/39	.28	...	1	.....
8/17/39	trace	...	4	Thin, feebly active
8/29/39	6.88	268++	6	Normal, heavy, highly active
Therapy discontinued for three weeks				
9/20/39	4.62	164+		Good, active
10/20/39	7.40	200+	11	Heavy, highly active

\* A young bull that became sterile shortly after entering service.

level of approximately 6.19 mg. per cent on the average. The appearance of ascorbic acid in the semen was accompanied by distinct changes in the character of the semen sample. Formerly, the semen was thin and watery and totally devoid of life. It had now become normal in appearance, thick and creamy, with every evidence of life and vitality. Ascorbic acid treatment was suspended for 22 days. Tests were again made and 2 grams of ascorbic acid given. Four, seven and eight days later 3 heifers were bred to this bull. One was bred with semen stored for 96 hours. The heifers were slaughtered three weeks after breeding. Embryos were found in two, but the one bred with the stored semen had not conceived.

Table 2 summarizes the data from a number of other bulls given ascorbic acid therapy. Several important facts are noticeable. There was a rise of

95 per cent in the ascorbic acid content of the blood plasma. There was a general movement of the ascorbic acid content of the semen toward a range lying between 3.00-8.00 mg. per 100 cc. of fresh semen. In our experience, completely impotent bulls have shown less than 2 mg. of ascorbic acid per 100 cc. of semen. On the other hand, bulls which had a questionable breeding record, or were only partially potent very often showed excessive amounts of ascorbic acid in the semen sample. There was little change in the ascorbic acid content of successive semen collections taken at one sampling although a slight drop was experienced. With the appearance of normal ascorbic acid values in the blood plasma and semen samples there was uniformly greater sperm vitality as evidenced by the longevity record. This is of special significance.

It will be noticed that the laboratory data on eleven of the bulls was incomplete. These bulls were treated under practical farm conditions. Frequently, herd sires used under natural breeding conditions would not allow semen collections by means of the artificial vagina, were untractable, or distance made it impossible to obtain the data directly. In the latter case veterinarians, county agents, or the authors gave the treatments and obtained reports on the breeding performance as recorded. Where semen samples were available the general characteristics were noted. Semen from seven of these bulls was observed grossly or microscopically, or both. In these cases the semen character was changed by the treatment from the thin aqueous type characteristic of low grade semen to the thick creamy highly motile semen of the normal bull. In this regard it was interesting to record the case of one Guernsey bull which failed to respond to ascorbic acid treatment. It had been noticed that the conversion of carotene might be involved in the impotency of certain bulls. With this observation in mind the above bull was given vitamin A in the form of high grade cod liver oil at the rate of 20 cc. per day. The ascorbic acid therapy was continued. The bull quickly returned to the production of a high grade potent semen which was equivalent to or better than seven other bulls maintained in that particular breeding cooperative association.

Some of the bulls had little sex interest and a few would not breed at all. Six were classified in this category, or were extremely slow breeders. These cases have shown a distinct "lift" in sex interest after therapy. They are again in service.

Two bulls have shown improvement followed by relapse a few weeks after the treatment was suspended. These cases responded again to the therapy after relapse. It seems that the regular heavy service demands upon them crowds them beyond their limits and exogenous sources of ascorbic acid are needed.

An attempt to feed the ascorbic acid by mouth and effect a change in the ascorbic acid content of the blood plasma was unsuccessful. A vicious hard-

TABLE 2  
*Partial summary of data on bulls treated by subcutaneous injections of ascorbic acid*

Bull	Ascorbic acid (mg. %)		Longevity in York buffer (hr.)		Total ascorbic acid administered	Type of case
	Semen	Blood plasma	Before	After		
	Before	After	Before	After		
121	5.36	7.44	.....	.44	0	125++
H	2.18	.....	.22	.62	.46+	Young bull rendered impotent by an infection
1230	4.74	5.24	.18	..	120++	5 yr. old show bull
D	6.40	5.66	.16	.36	192++	Young growing bull
C	5.20	6.20	.08	.26	120+	Heavily used mature bull
B	9.70	9.00	.20	.36	240++	“ “ “
V	7.60	6.20	.22	.34	140++	Young growing bull
Mc	0.82	.....	.30	.96	216+	Mature bull, poor potency
O	5.36	2.62	.32	.50	192+	Young mature bull (winter)
N	8.02	7.08	.18	..	15 cows impregnated	Mature heavily used bull
M	.....	.....	.20	.26	(15 cows impregnated)	Aged mature bull, lost interest and potency
W	8.10	.....	.14	..	96+	Young mature heavily used bull
Ave.	5.77	6.18	.20	.39	69.6+	8.0
Bulls	Before treatment		After treatment			
S, OI, OG, C, B, T, D, W, IP, LA	These bulls showed the following:		After treatment			
	1. Breeding slow, uncertain		1. Breeding improved, positive impregnation percentage increased			
	2. Semen poor, low motility		2. Semen good to excellent, motility active			
	3. Sex interest indifferent or nil		3. Sex interest stimulated and improved			
	4. Returned to service again					

to-handle bull was fed one gram of ascorbic acid daily for 6 days. Subsequent examination of the blood plasma indicated that there had been no change in the ascorbic acid level.

A total of twenty-nine bulls have been treated. Four of these have not responded to ascorbic acid therapy by any measurable means. Therefore, it appears that about four out of every five bulls treated have responded favorably to ascorbic acid injections. One of the bulls which did not respond has been unofficially reported to have developed testicular atrophy previous to treatment. Fifteen of the bulls used in these studies were definitely headed for the discard when they came to our attention. They are again back in regular service after ascorbic acid therapy.

#### DISCUSSION

Ascorbic acid therapy is not expected to cure all cases of sterility in the bull. These researches do indicate, however, that it was distinctly beneficial in certain types of sterility. It seems to be favorable when bulls are in the growing and developmental process, or in the cases where rather heavily used potent bulls begin to decline in ability to "settle."

At present it is impossible to suggest why this type of impotency should develop. Ascorbic acid has been shown to be present in the cells of the endocrine glands concerned in reproductive functions. This work has been excellently summarized in the monograph by Giroud (5). Improper feeding over long periods of time, developmental failure, injury or infection might be singly or collectively responsible for this type of sterility. Until the tissues or organs responsible for ascorbic acid synthesis are more clearly defined the cause of impotency due to ascorbic acid deficiency will of necessity remain obscure.

It seems that the ascorbic acid content of fresh semen, freshly drawn blood, and longevity of sperm in yolk buffer gives a fairly accurate rating of potency in the bull. A few samples of semen treated with H<sub>2</sub>S and then tested for ascorbic acid under nitrogen gave values very close to those for ascorbic acid alone. Whether there is a relationship between the ascorbic acid and dehydroascorbic acid in semen is not known at this time. In blood plasma certain field samples from outlying points have given abnormally high ascorbic acid values. Whether these values were caused by the liberation of bound ascorbic acid which is released upon standing is not known. This possibility seems unlikely in view of our present knowledge of ascorbic acid chemistry. Longevity of sperm in yolk buffer gives a fair degree of accuracy in rating potency in the bull. This test is easily applicable where semen samples are regularly collected. A poor breeding bull's semen will maintain motility for less than 100 hours while a potent bull semen sample will maintain motility for more than 200 hours. Thus, poor breeding or low potent semen stores for less than 100 hours, good semen will store for 100-

200 hours, and very potent semen will store with a high degree of activity for 200 hours and more.

#### SUMMARY AND CONCLUSIONS

These data indicate several important results: (1) the subcutaneous injection of ascorbic acid resulted in the restoration of the fertilizing capacity of certain impotent bulls; (2) potent bull semen normally contained on the average of 6.19 with a range of 3.0-8.0 mg. of ascorbic acid per 100 cc. of fresh semen; values below 2 mg. were associated with impotency, or poor breeding; (3) high ascorbic acid values, 8.0 mg. or more, on the other hand were associated with bulls with an unreliable breeding record; and (4) the ascorbic acid content of fresh semen, freshly drawn blood plasma and especially the longevity of sperm in yolk-buffer provides a fairly accurate estimate of potency or impotency in the bull.

From these data it is concluded that ascorbic acid is intimately involved in the production of virile sperm in the bull and in some manner it is vitally concerned in the physiology of reproduction in the male bovine. The exact nature of its role in this capacity is not known.

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THE GAS REQUIREMENTS OF MOLDS. II. THE OXYGEN REQUIREMENTS OF *PENICILLIUM ROQUEFORTI* (THREE STRAINS ORIGINALLY ISOLATED FROM BLUE VEINED CHEESE) IN THE PRESENCE OF NITROGEN AS DILUENT AND THE ABSENCE OF CARBON DIOXIDE

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INTRODUCTION

The object of this study was to determine to what extent the O<sub>2</sub> of the air must be diluted with N<sub>2</sub> to show the inhibition of growth of strains of *Penicillium roqueforti*.

The previous study (4) has shown the marked difference in the growth of strains of *P. roqueforti* in an atmosphere diluted with N<sub>2</sub> as compared with CO<sub>2</sub>. The study was continued with the object of determining the O<sub>2</sub> requirements of strains of *P. roqueforti* in CO<sub>2</sub>-free air, diluted with N<sub>2</sub>, over a wide range of temperature. The CO<sub>2</sub> in the air and that produced by the mold during growth were absorbed by NaOH solutions, to produce as far as possible the absence of CO<sub>2</sub> during growth.

LITERATURE

Since writing the previous paper (4) of this series, little work has been published on the O<sub>2</sub> requirements of any of the molds in the absence of CO<sub>2</sub>. Certain new work refers to the need for O<sub>2</sub> in gluconic acid production by *Aspergillus niger* (6), but since, in the method described, the acid formed is neutralized by precipitated chalk (CaCO<sub>3</sub>) it is questionable whether the presence of CO<sub>2</sub> is not the more important factor, which necessitates a frequent change of air during the fermentation.

CULTURES

Three cultures of blue mold, strains of *P. roqueforti*, from the previous study (4) were used, namely:

Culture 32. Isolated from a Wensleydale cheese made at the University of British Columbia.

Culture 33. Similar origin.

Culture 37. Isolated from a Wensleydale cheese made by Rowntree, York.

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Studies of their cultural characteristics and morphology have been previously reported (2, 3).

*Purification.* Before commencing the study the cultures were repurified. The usual poured-plate technique was used. The medium employed was very clear whey gelatin, which, with the assistance of a low power binocular microscope, enabled the marking of the colonies soon after germination. When the marked colonies had grown somewhat, they were transferred to agar slants. This was repeated four times. While it was not possible to select individual spores, it is reasonable to conclude that each culture was obtained from the growth of one or more spores of the same origin.

#### MATERIALS AND METHODS

*Medium.* From the results in the previous study it was decided to use Difco malt agar. Thus, to prevent changing the medium during the experiment, 17 lbs. of dehydrated Difco malt agar was purchased and stored at approximately 40° F.

*Slants.* Agar slants of the above medium were used for carrying the cultures and also for making the water dispersion of the mold culture to inoculate the plates. When required for water dispersion, the inoculated agar slants were grown for 11 days at from 65° F. to 70° F.

*Preparation of plates.* As in the previous work (4), 25 ml. of the above medium were used for each plate. After pouring, the plates were put on a cold slab to solidify, which prevented condensation on the lids.

*Inoculation of plates.* One ml. of sterile water was added to the 11-day-old slant culture with a sterile pipette, and after mixing it was returned to a 100 ml. water blank and shaken well. A sterile "L"-shaped platinum wire was dipped into the water dispersion of mold spores and used to inoculate the center of the plate. Thus the colony started from a small hole of fairly uniform size made by the platinum "L." Examination under the binocular microscope showed that growth started from an area of 2 mm. or less. The same wire was used in all cases. For each growth curve determination, 5 plates for each culture at each temperature of incubation were inoculated.

*Incubation.* The same battery of incubators was used as in the previous study (4). Seven of these compartments were set to range in temperature at about equal intervals for a total variation of from 46° F. to 90° F. A thermograph or maximum and minimum thermometer was kept in each compartment to determine fluctuations in temperature. Also, 2 thermometers for each compartment were sealed with wax in small bottles containing water and read and recorded twice daily. The average of the readings of the latter was considered as the temperature of the incubator for the period of incubation. The slight variations in temperature from day to day seldom exceeded 1° F. In the later experiments Weston metallic thermometers were fitted into the desiccators and gave the temperature directly in contact with the plates.

*Nitrogen.* In this study, N<sub>2</sub> was the only gas used to dilute the air. The N<sub>2</sub> was used from the same commercial cylinder of gas.

*Gas chamber.* As in the previous study (4) 250-mm. Pyrex vacuum desiccators were used as the gas chambers for growing the molds in all cases. The maximum capacity of these desiccators is 22 Petri dishes each, when using only the space above the desiccator plate. One desiccator was put in each incubator compartment and connected with 2 glass tubes. One tube was connected to a common gas line to a mercury manometer, a suction pump, and the nitrogen cylinder. The other gas tube was the air intake for each desiccator, which was connected to 4 gas bottles (specially-fitted quart milk bottles). The gas bottles, which were held at the same temperature as the desiccator, were in the following order: First, a bottle of a 10 per cent solution of NaOH to remove CO<sub>2</sub> from the air; second, a bottle of dilute solution of H<sub>2</sub>SO<sub>4</sub> to prevent any NaOH being carried over into the last 2 bottles, which contained a saturated solution of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and its crystalline salt; the last bottle was connected to the desiccator. Thus the air drawn into the desiccator passed through NaOH to remove CO<sub>2</sub>, then through H<sub>2</sub>SO<sub>4</sub>, and finally through a saturated solution of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to humidify the air to about 80 per cent and prevent the desiccation of the medium on the plates.

Experimentally, it was found that over a period of 7 days (the 10 per cent added N<sub>2</sub> growth curve) a plate lost not more than 1.0 gram at 87° F. or about 0.5 gram at 60° F. To remove CO<sub>2</sub> from the air in the desiccator, a porridge dish containing 5 per cent NaOH was held in the space below the desiccator plate.

During the growth period there was no need to move the desiccator from the incubator for changing the gas supply. Thus, variations in changes in temperature were avoided. Furthermore, the desiccator and gas wash bottles were held in their respective incubators before the plates were added, thus shortening the time for adjusting the temperature of the inoculated plates.

*Adding and changing gases.* The desiccators, all having been filled with the required inoculated plates, were simultaneously evacuated to reduce the content of the air to the required fraction. The reduced pressure was measured with the manometer. The N<sub>2</sub> was then added to the desiccators until atmospheric pressure was reached.

Example: Required: a mixture of 30 per cent of air and 70 per cent N<sub>2</sub>. Barometer pressure 700 mm. The desiccators were all simultaneously evacuated to a column of mercury of  $\frac{700 \times 70}{100} = 490$  mm. N<sub>2</sub> was then added to atmospheric pressure.

A daily change of gas was made simultaneously in all desiccators by bubbling air briskly through the solution in the 4 gas bottles and on through

each desiccator for 10 minutes. Thus, natural air free from CO<sub>2</sub> and at the specified humidity was obtained around all the plates in the desiccators. To change the gas over the plates, the desiccators were evacuated to 500 mm. back pressure and returned to atmospheric pressure via the gas bottles. This was repeated in all 3 times. When the molds were required to grow in a gas supply other than air free from CO<sub>2</sub>, the desiccators were simultaneously evacuated to the required pressure (see above example) and then filled to atmospheric pressure. Both outlet and inlet to the desiccators were then closed; thus, only very slight deviation from atmospheric pressure occurred. The daily gas change took from 40 to 60 minutes. Being repeated 7 times for each growth curve of 168 hours, an unavoidable error of 4 to 5 hours growing in air is introduced in each growth curve.

*Growth period.* After inoculation, which required less than one hour, the plates were inverted and put in their respective desiccators and incubated for a period of 7 days.

*Measurement of colony.* Wherever possible, the growth of the colony of mold was expressed in millimeters representing the average diameter of 5 colonies. (There were very few exceptions where the organisms either failed to grow or became contaminated, in which case the average diameter was obtained from less than 5 colonies.)

*Expression of growth.* From the above measurements, curves have been drawn for each change in gas supply, using millimeters growth as the vertical axis and temperature as the horizontal axis. Such curves have the advantage of permitting:

1. The making of a control curve for all temperatures of growth. Thus, controls do not have to be run concurrently with each curve made under changed gas supply.
2. The interpolation of the average size of colony for any temperature over the range of growth for the culture.
3. By interpolation the plotting of a growth curve for any temperature, having growth as the vertical axis and concentration of any gas at the horizontal axis.
4. The capacity of the culture of mold to grow under a definite condition, to be expressed by the area enclosed by the curve. (The areas were expressed in the same units.)
5. The expression of growth in air, less CO<sub>2</sub>, on the basis of 100 for all temperatures. Thus a comparison can be made with the growth of the same culture grown under any other gas supply at the same temperature, and in the same medium.

*Example:* Culture 32 D grown at 70° F. showed from the growth curve by interpolation a diameter of 55 mm. in air less CO<sub>2</sub>. Culture 32 D grown at 70° F. showed from the growth curve by interpolation a diameter of 48 mm. in air less CO<sub>2</sub> 20 per cent, added N<sub>2</sub> 80 per cent.

Thus, as  $55 : 48 = 100 : x$

$$x = \frac{48 \times 100}{55} = 87.3$$

NOTE: The method permits of seeing at once the percentage of variation on a common basis for all cultures and for any temperature of growth. Comparisons with many other organisms, which are adapted to the technique, can later be made.

Also, insignificant variations (i.e., not exceeding 10 per cent from the control) are immediately apparent.

#### PRELIMINARY EXPERIMENT

An experiment was conducted according to the method already described to develop 3 control curves, namely:

1. Air less CO<sub>2</sub> (Approximately 21 per cent O<sub>2</sub>).
2. Air with no CO<sub>2</sub> removed (Approximately 21 per cent O<sub>2</sub>).

3. Air less CO<sub>2</sub> evacuated to 90 per cent vacuum to determine whether a high vacuum would effect the mold growth (Approximately 21 per cent O<sub>2</sub>).

The curves are given in figures 1, 2, and 3 for the cultures 32 D, 33 D, and 37 D respectively; also in table 1. The curves for each culture show that there is not a significant difference of over 10 per cent except for curve 3 which is slightly irregular at high temperatures. The curves 1 (air less

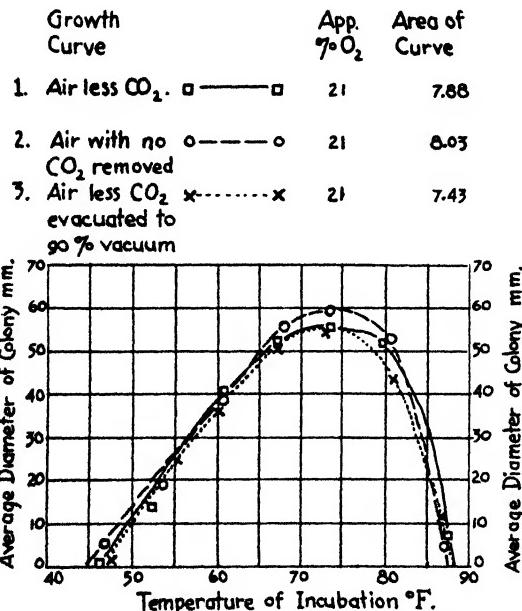


FIG. 1. Control curves to determine the significance of different techniques. Culture 32 D.

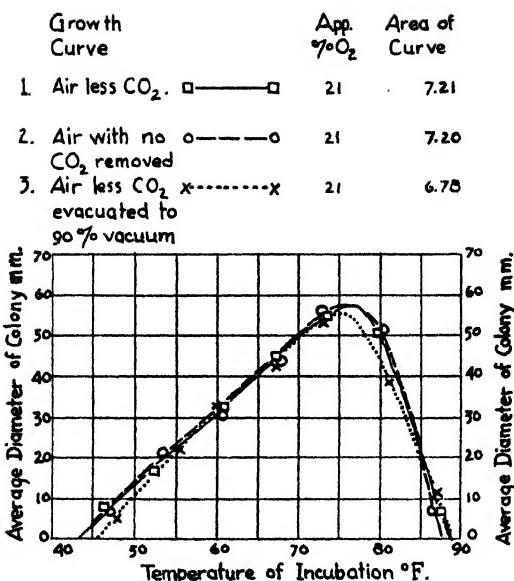


FIG. 2. Control curves to determine the significance of different techniques. Culture 38 D.

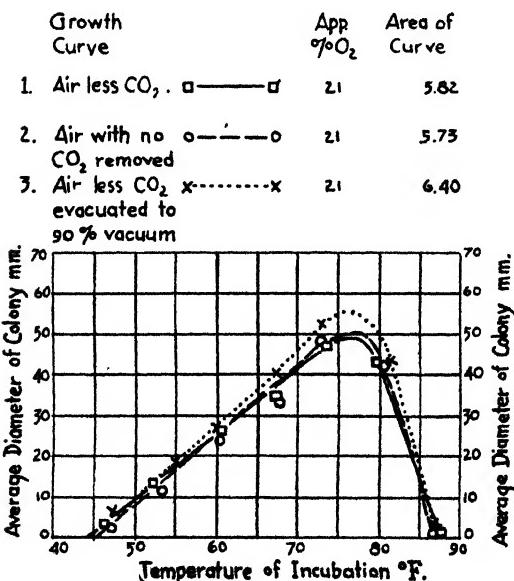


FIG. 3. Control curves to determine the significance of different techniques. Culture 37 D.

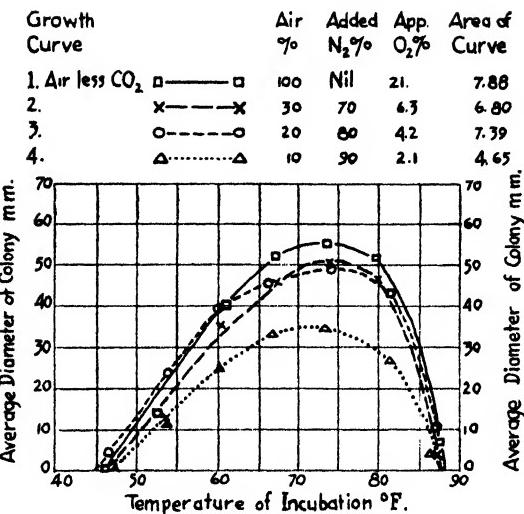


FIG. 4. The effect on the growth of *P. roqueforti* by the reduction of oxygen obtained by adding nitrogen, culture 32 D.

CO<sub>2</sub>) and 2 (air with no CO<sub>2</sub> removed) for all 3 cultures nearly superimpose and would justify using either as control. In all subsequent experiments curve 1 (air less CO<sub>2</sub>) was used as the control for comparison, with the other curves having a reduced O<sub>2</sub> supply in the absence of CO<sub>2</sub>.

Growth Curve	Air %	Added N <sub>2</sub> %	App. O <sub>2</sub> %	Area of Curve
1. Air less CO <sub>2</sub>	100	Nil	21.	7.21
2.	x—x	30	70	6.22
3.	o—o	20	80	4.48
4.	△—△	10	90	4.79

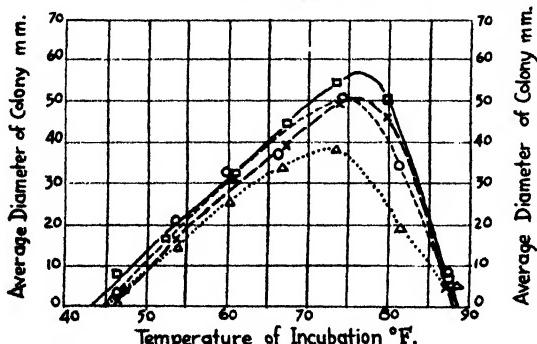


FIG. 5. The effect on the growth of *P. roqueforti* by the reduction of oxygen obtained by adding nitrogen. Culture 33 D.

Growth Curve	Air %	Added N <sub>2</sub> %	App. O <sub>2</sub> %	Area of Curve
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1. Air less CO <sub>2</sub>	100	Nil	21.	5.82
2.	30	70	6.5	5.15
3.	20	80	4.2	3.57
4.	10	90	2.1	4.08

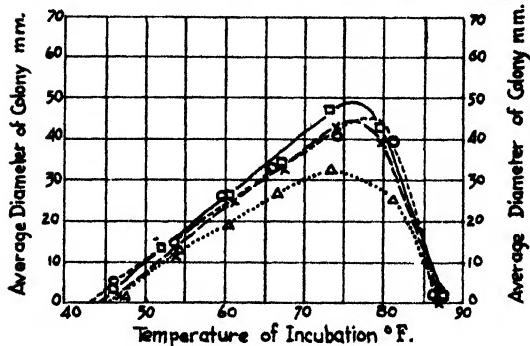


FIG. 6. The effect on the growth of *P. roquefortii* by the reduction of oxygen obtained by adding nitrogen, culture 37 D.

#### EXPERIMENTAL

The oxygen requirements in the presence of N<sub>2</sub> as diluent and the absence of CO<sub>2</sub>.

Figures 4, 5, and 6, growth curves for cultures 32 D, 33 D, and 37 D, respectively, show the seven-day growth curves when the air less CO<sub>2</sub> has

#### Temperature of Growth.

55°F. □—□      70°F. ×—×  
60°F. ○—○      80°F. △—△

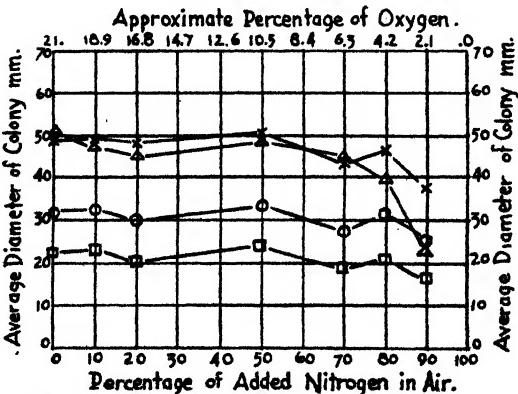


FIG. 7. The effect on the growth of *P. roquefortii* by the reduction of oxygen obtained by adding nitrogen. Culture 33 D.

TABLE 1

*Acceleration or reduction in growth of cultures of *P. roqueforti* resulting from a change of gas supply. Expressed on the basis of growth in air less CO<sub>2</sub> at the same temperature as 100. Seven days' growth*

Curve	Gas supply by volume			Culture	Temperature				
	Air % CO <sub>2</sub>	Added % N <sub>2</sub>	Approximate % O <sub>2</sub>		55° F.	60° F.	70° F.	80° F.	Area
1	100 less CO <sub>2</sub>	Nil	21	32 D	100	100	100	100	100
				33 D	100	100	100	100	100
				37 D	100	100	100	100	100
2	100	Nil	21	32 D	106	97	105	102	102
				33 D	102	103	100	100	100
				37 D	91	100	102	102	98
3	*100 less CO <sub>2</sub>	Nil	21	32 D	96	92	100	90	94
				33 D	96	103	100	86	94
				37 D	103	108	112	117	110

\* Evacuated to the same pressure as for 90% N<sub>2</sub>.

TABLE 2

*Acceleration or reduction in growth of cultures of *P. roqueforti* resulting from a change of gas supply. Expressed on the basis of growth in air less CO<sub>2</sub> at the same temperature as 100. Seven days' growth*

Curve	Gas supply by volume			Culture	Temperature				
	Air % (Less CO <sub>2</sub> )	Added % N <sub>2</sub>	Approximate % O <sub>2</sub>		55° F.	60° F.	70° F.	80° F.	Area
1	100	Nil	21	32 D	100	100	100	100	100
				33 D	100	100	100	100	100
				37 D	100	100	100	100	100
	90	10	18.9	32 D	108	100	100	100	102
				33 D	104	110	102	98	98
				37 D	97	104	107	105	103
	80	20	16.8	32 D	110	103	104	100	103
				33 D	89	97	98	92	92
				37 D	94	100	100	93	97
	50	50	10.5	32 D	108	103	98	92	99
				33 D	109	110	104	102	105
				37 D	100	100	98	98	100
2	30	70	6.3	32 D	84	85	89	88	86
				33 D	84	90	90	90	86
				37 D	89	92	93	90	88
3	20	80	4.2	32 D	108	102	87	87	94
				33 D	96	103	96	80	90
				37 D	94	96	90	102	96
4	10	90	2.1	32 D	64	64	64	56	59
				33 D	76	84	76	46	66
				37 D	72	76	73	64	70

been diluted with 70, 80, and 90 per cent N<sub>2</sub> respectively, as compared with the control air less CO<sub>2</sub>. The additional seven-day growth curves of the 3 cultures when the air less CO<sub>2</sub> has been diluted with 50, 20, and 10 per cent N<sub>2</sub> respectively are not given as they superimpose with the control. However, these results obtained from the curves by interpolation are shown in figure 7 for culture 33 D.

Figure 7, for culture 33 D, was plotted by interpolating the points from the seven-day growth curves for temperatures of 55, 60, 70, and 80° F. and show by another expression the effect of reduced oxygen supply by adding N<sub>2</sub> in the absence of CO<sub>2</sub> on growth.

Table 2 shows the acceleration or reduction in growth of the cultures on a percentage basis, resulting from a reduction in O<sub>2</sub> by the addition of N<sub>2</sub> to the gas supply.

Together, the growth curves, figures 4, 5, and 6, with growth plotted against concentration of gas, figure 7 and table 2 in which growth is expressed on a percentage basis show:

1. A very low O<sub>2</sub> concentration, in the order of less than 4.2 per cent, is required before growth can be significantly reduced.\*
2. There is a definite trend for the reduction in growth—caused by low O<sub>2</sub>, shown in curves 5 and 6—to be proportionately greater above the optimum temperature of growth than that below the optimum.
3. Where temperatures below 55° F. are used, the period of 7-day growth is not sufficient to obtain large enough colonies for a good comparison.
4. The 3 strains of mold used show little difference in their response to the inhibiting effect of very low concentrations of O<sub>2</sub>. However, it is fairly definite that culture 33 D is most affected above the optimum temperature of growth, while it is probably least affected below optimum temperatures.

#### DISCUSSION

The data in this paper agree with the data presented in the previous paper (4) insofar as the work is comparable. The length of the growth period in the previous study (4) was longer at the low temperatures of growth and the methods of adding the gas and handling the controls were sufficiently different to account for the small variations recorded. The data in this paper which indicate that no appreciable effect in growth is shown until very low concentrations of O<sub>2</sub> are reached are in agreement with the findings of Brown (1) using *P. glaucum* and *Fusarium* sp.

The reduction in growth of *P. roqueforti*, which is recorded by Thom and Currie (5), cannot in the least be attributed to low concentrations of O<sub>2</sub>, which they obtained by adding CO<sub>2</sub>.

\* It was observed that the appearance of the colonies was not in the least changed, except in size, between the range of 21 and 2.1 per cent of O<sub>2</sub> when N<sub>2</sub> was used as the diluent of the air.

It would seem that the reduction of growth brought about by lowering the O<sub>2</sub> supply in the presence of N<sub>2</sub> first occurred at the high temperatures of growth. The cause of this has not been determined. However, should this be a function of the absorption coefficient of the gas (O<sub>2</sub>), the problem of whether it is associated with the medium or the moisture content in the mold itself will have to be determined. Whatever the cause, there is no question that this part of the investigation still presents a valuable and interesting physiological study.

#### CONCLUSION

1. Three strains of blue mold (*P. roqueforti*) from cheese have been grown at 7 different temperatures in atmospheres of from 21 per cent to 2.1 per cent O<sub>2</sub>, obtained by adding N<sub>2</sub> to the air. The results are expressed graphically.
2. It was only with the greatest O<sub>2</sub> dilution (2.1 per cent O<sub>2</sub>) that a significant reduction of growth was recorded. This ranged between 16 per cent and 54 per cent.
3. It would appear that there is a tendency for this same shortage of O<sub>2</sub> to reduce growth more at the higher temperatures.

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THE GAS REQUIREMENTS OF MOLDS. III. THE EFFECT OF  
VARIOUS CONCENTRATIONS OF CARBON DIOXIDE ON  
THE GROWTH OF *PENICILLIUM ROQUEFORTI*  
(THREE STRAINS ORIGINALLY ISOLATED  
FROM BLUE-VEINED CHEESE) IN AIR\*

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INTRODUCTION

This study is a continuation of previous studies (6, 7) to determine the significance on the growth of strains of *P. roqueforti* of various concentrations of CO<sub>2</sub> in the air at different temperatures. It was considered that the previous study (6) did not fully cover the range of possible dilutions of air with CO<sub>2</sub>, or the range of the temperatures of growth of the strains of *P. roqueforti* used.

Therefore, the object of this study was to determine to what extent the addition of CO<sub>2</sub> to air affects the growth of strains of *P. roqueforti* at various temperatures of growth.

The complementary paper to this study "The oxygen requirements of *Penicillium roqueforti* in the presence of nitrogen as diluent, and the absence of carbon dioxide" (7) reported experiments conducted with practically identical methods. Thus, the results are directly comparable with one another and permit a close estimate of the relative significance of various concentrations of oxygen, nitrogen and carbon dioxide on the growth of *P. roqueforti* at different temperatures.

LITERATURE

Since the publication of the previous paper (6), no work has been published on the gas requirements of *P. roqueforti*. However, a great deal of work has been done on the effect of gas storage—mainly increased concentrations of CO<sub>2</sub>—on fruit (1, 3, 4, 5), eggs (8, 9), and meat (9, 10). These workers were chiefly concerned with the practical use of CO<sub>2</sub> in gas cold storage, and while several reported reduction in mold growth by gas cold storage as compared with the control, little has been recorded of the species of mold which is inhibited by the various concentrations of CO<sub>2</sub> used. With the exception of Eaves (3), who worked with *P. expansum*, no attempt has been made to work with material which was inoculated with a known culture of mold. It would seem that if the inhibition of mold growth by CO<sub>2</sub> cold

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storage is to be regularly accomplished, a more systematic approach to the problem must be undertaken. It is hoped that the procedures used in this and previous papers (6, 7) may be of some assistance in this respect.

Acceleration in growth caused by low concentrations of  $\text{CO}_2$  on the growth of plant tissue has been observed (3, 12). Therefore, it has been considered desirable to include in this investigation the effect on the growth of *P. roqueforti* at various temperatures of low concentrations of  $\text{CO}_2$  in air.

#### MATERIALS AND METHODS

The materials and methods are identical with those of the previous paper (6) except for the following:

*Gas.* Carbon dioxide was used direct from a commercial cylinder of the gas.

*Gas chamber.* The same Pyrex vacuum desiccators were used for these experiments as in the previous work (7). They were operated in the same manner with the exception that no  $\text{NaOH}$  solutions could be used to remove  $\text{CO}_2$ . Therefore, the first and second gas bottles (specially-fitted quart milk bottles) were filled with very dilute mercuric chloride and a saturated

#### GAS MIXTURE

Air % CO <sub>2</sub>	Added % CO <sub>2</sub>	App. N <sub>2</sub> %	App. O <sub>2</sub> %	Area of Curve
○—○ 100	Nil	79.	21.	8.03
□—□ 90	10	71.1	18.9	9.15
○—○ 60	40	47.4	12.6	5.19
X—X 30	70	23.1	6.3	3.12
Δ—Δ 20	80	15.8	4.2	1.82

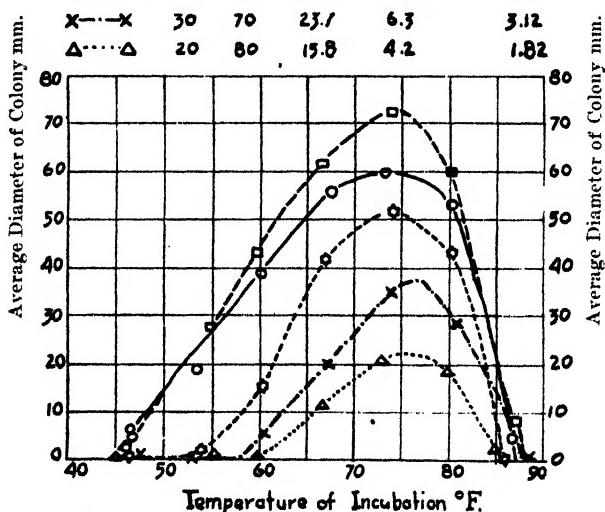


FIG. 1. The effect on the growth of *P. roqueforti* by the reduction of oxygen obtained by adding carbon dioxide, culture 32 D.

$(\text{NH}_4)_2\text{SO}_4$  and its crystalline salt respectively in place of the  $\text{NaOH}$  solution and the dilute solution of  $\text{H}_2\text{SO}_4$  previously used. Thus, 4 gas bottles were connected in line so that air entering the desiccator would be maintained at constant humidity and temperature.

*Adding and changing gases.* The method of changing and adding gases to the desiccators was identical with that of the previous study (7) except for the substitution of  $\text{CO}_2$  for  $\text{N}_2$ .

#### EXPERIMENTAL

Five growth curves have been selected and are given in figures 1, 2, and 3 for the 3 cultures used. These curves have been chosen from the 12 curves used in preparing figure 4, and table 1 as they show:

1. The control curve is identical with curve 2 in the previous paper (7) and practically superimposes curve 1 (7) in that paper and was used as the control air less  $\text{CO}_2$ .
2. The maximum increase in growth for each culture, obtained by relatively small additions of  $\text{CO}_2$ , has been selected for each culture on the basis of maximum area, table 1.

#### GAS MIXTURE

Air % CO <sub>2</sub>	Added % CO <sub>2</sub>	App. N <sub>2</sub> %	App. O <sub>2</sub> %	Area of Curve
○—○ 100	Nil	79.	21.	7.20
□—□ 80	20	63.2	16.8	8.51
◇—◇ 60	40	47.4	12.6	5.70
×—× 30	70	23.7	6.3	3.43
△—△ 20	80	15.8	4.2	2.11

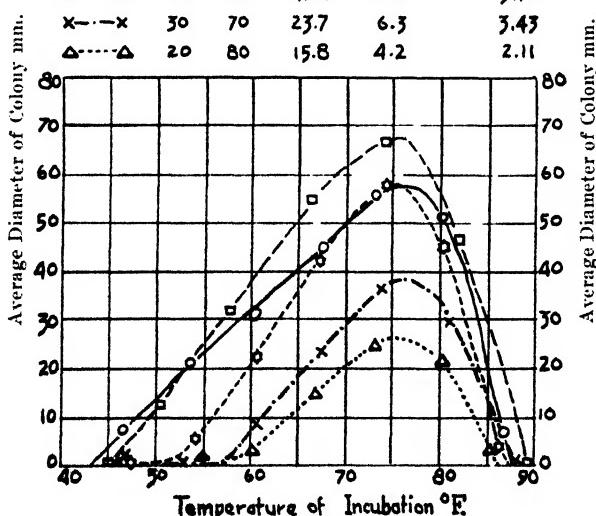


FIG. 2. The effect on the growth of *P. roqueforti* by the reduction of oxygen obtained by adding carbon dioxide, culture 33 D.

## GAS MIXTURE

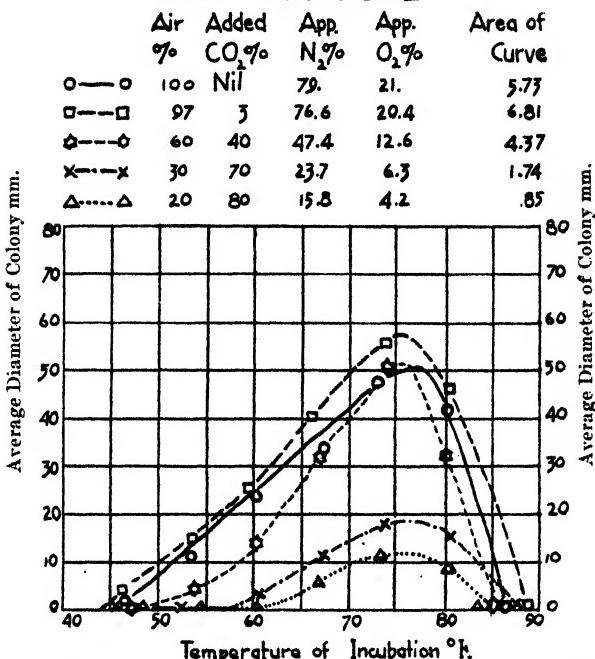


FIG. 3. The effect on the growth of *P. rugosafaciens* by the reduction of oxygen obtained by adding carbon dioxide, culture 37 D.

3. The remaining 3 curves show the gradual reduction in growth for all 3 cultures by increasing the CO<sub>2</sub> content of the air.

Figures 1, 2, and 3, the actual growth curves; figure 4, obtained by interpolation from the 12 growth curves; and table 1, the interpolated results expressed on a percentage basis, each show in their particular way:

1. Dilute concentrations of CO<sub>2</sub> in air increase the mold growth.
2. High concentrations of CO<sub>2</sub> in air inhibit or even prevent mold growth.
3. The increase or decrease in growth caused by CO<sub>2</sub> is a function of temperature. Thus table 1 shows maximum increases in growth at 50° F. for all cultures occurring at 3 or 7 per cent CO<sub>2</sub>, at 60 and 70° F., maximum growth occurs at 10 per cent or 17 per cent CO<sub>2</sub>, and at 80° F. maximum growth occurs at 20 per cent or 30 per cent CO<sub>2</sub>. Figures 1, 2, 3, and 4, show clearly how the inhibition of growth of the molds by CO<sub>2</sub> first occurs at the low temperatures and later at the higher temperatures as the concentrations of CO<sub>2</sub> are increased. Thus it is seen that at or above 50 per cent CO<sub>2</sub> little or no growth can be expected at 50° F., while growth at 60° F. is of the order of 50 per cent.

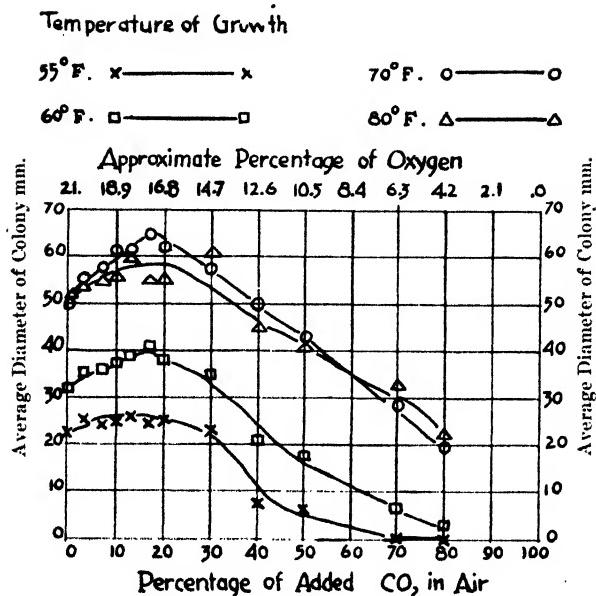


FIG. 4. The effect on the growth of *P. roqueforti* of carbon dioxide in air, culture 33 D.

4. Using the area of the 3 cultures as given in table 1, as an index of their growth response to CO<sub>2</sub>, it is shown that culture 33 D requires a greater concentration of CO<sub>2</sub> for maximum acceleration of growth. Also, the same culture at high concentrations of CO<sub>2</sub> is less inhibited in growth by CO<sub>2</sub> than the other 2.

#### DISCUSSION

This work is in complete agreement with the first paper (6) as far as inhibiting effect of high percentages of CO<sub>2</sub> on the mold growth were observed. That CO<sub>2</sub> is much more effective in inhibiting mold growth at low temperatures is again shown with more extensive data, which is in agreement with the findings of Brown (2) who used ordinary fruit-rot organisms such as *Botrytis*, *Fusarium*, and *Alternaria*.

The acceleration of mold growth by small percentages of CO<sub>2</sub> is definitely shown. This acceleration of growth is a function of temperature and occurs at the lower temperature of growth before it does at the higher temperatures of growth. Thus 30 per cent CO<sub>2</sub> in air shows definite acceleration in growth at 80° F. while the same concentration shows quite definite inhibition at 50° F. Therefore, it is obvious that the optimum and minimum temperatures of growth will be changed by the percentage of CO<sub>2</sub> in the gas supply.

Though these general principles apply to all 3 strains of *P. roqueforti*, it is definite that the strains are not equally affected. Culture 33 D is less

TABLE 1

*Acceleration or reduction in growth of cultures of *P. roqueforti* resulting from a change of gas supply. Expressed on the basis of growth in air at the same temperature as 100. Seven days growth*

Air %	Added CO <sub>2</sub> %	Gas supply by volume		Culture	Temperature				Area		
		Approximate			50° F.	60° F.	70° F.	80° F.			
		N <sub>2</sub> %	O <sub>2</sub> %								
100	Nil	79.0	21.0	32 D	100	100	100	100	100		
				33 D	100	100	100	100	100		
				37 D	100	100	100	100	100		
97	3	76.6	20.4	32 D	100*	111	114	104	113		
				33 D	107	113	110	108	110		
				37 D	143	100	119	112	119		
93	7	73.5	19.5	32 D	79	105	110	109	106		
				33 D	86	109	116	112	109		
				37 D	157	100	112	102	110		
90	10	71.1	18.9	32 D	100	116	117	115	114		
				33 D	86	116	122	112	116		
				37 D	129	108	121	102	113		
87	13	68.7	18.3	32 D	50	97	110	111	101		
				33 D	86	122	122	120	117		
				37 D	86	96	114	93	105		
83	17	65.6	17.4	32 D	21	105	109	104	98		
				33 D	71	128	130	108	117		
				37 D	114	112	126	93	117		
80	20	63.2	16.8	32 D	71	97	104	106	103		
				33 D	86	122	124	110	118		
				37 D	100	108	117	116	111		
70	30	55.3	14.7	32 D	64	95	107	117	105		
				33 D	71	109	114	124	113		
				37 D	14	84	107	102	96		
60	40	47.4	12.6	32 D	0	37	83	81	65		
				33 D	7	66	100	90	79		
				37 D	14	52	95	77	76		
50	50	39.5	10.5	32 D	0	53	74	85	68		
				33 D	0	56	86	84	73		
				37 D	0	40	67	58	57		
30	70	23.7	6.3	32 D	0	11	45	57	39		
				33 D	0	22	58	66	48		
				37 D	0	8	33	37	30		
20	80	15.8	4.2	32 D	0	3	28	36	23		
				33 D	0	9	40	44	29		
				37 D	0	0	24	23	15		

\* Italicized numbers indicate greatest acceleration of growth.

quickly accelerated by low concentrations of CO<sub>2</sub>. The latter is in agreement with the previous work (6).

## DISCUSSION IN RELATION TO THE OXYGEN REQUIREMENTS

Table 1 in the previous paper (7) shows no significant reduction of growth for 4.2 per cent O<sub>2</sub> where N<sub>2</sub> is the only diluent. The present paper shows a reduction in growth of over 75 per cent for the same concentration of O<sub>2</sub> where CO<sub>2</sub> is present to the extent of 70 per cent. Thus it is justified to conclude that except in very low concentrations of O<sub>2</sub>, CO<sub>2</sub> was the inhibiting factor and not the lack of O<sub>2</sub>. The reduction in growth of *P. roqueforti*, which is recorded by Thom and Currie (5), must be entirely attributed to the high concentration of CO<sub>2</sub> which was used to dilute the air rather than the low concentration of O<sub>2</sub>. The effect of temperature shown in the previous paper (7) where O<sub>2</sub> and N<sub>2</sub> are the only gases present, indicates that the reduction in growth of the molds, which can only be attributed to low concentrations of O<sub>2</sub>, occurs first at the high temperatures of growth where O<sub>2</sub> would be less soluble in the medium. These data show that the effect of CO<sub>2</sub> on growth is most noticeable at the low temperature of growth where the CO<sub>2</sub> is more soluble in the medium. Neither paper presents sufficient data to justify an attempt at correlation with the absorption coefficient of O<sub>2</sub> or CO<sub>2</sub> respectively. However, the possibilities of there being such a correlation would seem to be probable.

## CONCLUSIONS

1. Relatively small concentrations of CO<sub>2</sub> in air increase the growth of strains of *P. roqueforti* while large concentrations inhibit the growth.
2. With the same organism the acceleration of growth due to low concentrations of CO<sub>2</sub> takes place sooner at the lower temperature of growth than at the higher temperature.
3. With the same organism the inhibition of growth, due to large concentrations of CO<sub>2</sub> in air, is apparent at the low temperatures sooner than at the higher.
4. The different strains of *P. roqueforti* used show the same trend but have different tolerance to CO<sub>2</sub>.
5. Culture 33 D was the least affected by the action of CO<sub>2</sub>.

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# OBSERVATIONS ON THE GROWTH RESPONSES OF STREPTOCOCCUS LACTIS IN MASTITIS MILK<sup>1</sup>

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Several investigators have ascribed certain failures in the manufacturing of cheddar cheese to the presence of mastitis milk. Leitch (1) is of the opinion that mastitis infection should be suspected when recurring difficulties are experienced because of slow acid development, curd weakness and faulty texture. He noted also that the inclusion of even small amounts of mastitis milk in the cheese vat resulted in almost complete suspension of the desired lactic acid development. Whitehead and Cox (2) found that milk containing leucocytes in excess of 5,000,000 per ml. gave rise to a rennet curd in which streptococci were not able to develop normal amounts of acid. Davis and Mattick (3) concluded that visibly abnormal milk should be excluded in the manufacturing of cheese and that milk reacting positively to the strip cup or to the bromoresol-purple test should be regarded as being abnormal until proof is obtained that it may be used with safety. Davis (4, 5) states that slow starter is still the most common fault in cheese making and one of the most serious consequences of mastitis. He is of the opinion that this condition, together with other factors induced by mastitis, causes more trouble than is realized in the making of cheese. According to Davis (6) the most common cause of slow starters in England appears to be abnormal milk from mastitis afflicted cows. He cites four changes in the milk that may influence the rate of growth of starter organisms, namely: (a) changes in the chemical composition of the milk, particularly a decrease in the lactose, casein, calcium and acidity; (b) changes in some enzymes and decreases in some vitamins and bacterial growth factors; (c) increased number of bacteria in the udder; and (d) apparent production, in rare instances, of substances strongly toxic to starter organisms.

Davis and McClemont (7) studied the acid coagulating time in both normal and mastitis milk. With the majority of the mastitis milk samples slow growth of *S. lactis* and *S. cremoris* occurred, whereas most of the normal samples, but not all, supported normal growth of these organisms. They consider that the reason for the slow growth of these organisms, probably, is due to the abnormal chemical composition of such milk.

In the present study frequent observations, over an extended period of time, were made on the growth responses of *Streptococcus lactis* in milk drawn from the individual infected and non-infected udder-quarters of the

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same cows. The infection of some of these udder-quarters was of such a nature that the milk was definitely abnormal at some observation periods and apparently normal at other times.

The experimental data presented include only a portion of that collected, being confined to a study of the milk from three cows, but they are representative of the findings with milk from other animals.

#### PROCEDURE

Samples were collected from the individual udder quarters in such a manner as to exclude gross contamination, and each consisted of approximately 200 ml. of milk drawn after the first few streams had been discarded, except in the case of several of the very abnormal milks where it was not possible to obtain this amount at a sampling period. Portions of the milk were inoculated with a culture of *S. lactis*, incubated at 18° to 20° C. and observations made on subsequent growth. The rate of growth was measured by the changes in pH value using the quinhydrone electrode supplemented by microscopic examinations of stained preparations.

#### EXPERIMENTAL RESULTS

Of the three cows used in this study, cow 114 gave milk from one udder-quarter that was abnormal at all times, whereas the milk from the diseased udder-quarters of cows 126 and 118, as judged by its pH values and physical state, fluctuated between an apparently normal state and a definitely abnormal condition throughout the period of observation.

A summary of the results, as measured by changes in pH values, of numerous series of milk from each animal is shown in table 1.

TABLE 1  
*Range in pH values of milk from diseased and normal quarters before and after inoculation with *S. lactis**

Cow	Quarter	Condition of quarter	No. of samples	Range in pH		Range in pH after 24 hrs. incubation	
				Minimum	Maximum	Minimum	Maximum
114	LF	Diseased	14	7.10	7.67	6.44	7.30
	RF-RH and LH	Normal		6.54	6.78	4.41	5.23
126	RF	Diseased	20	6.54	7.30	4.54	7.05
	LF	Diseased		6.55	7.47	4.53	7.35
	RH and LH	Normal		6.49	6.78	4.52	5.14
118	RF	Diseased	22	6.68	7.30	4.68	7.10
	RH	Diseased		6.66	7.05	4.70	7.04
	LH and LF	Normal		6.49	6.82	4.30	5.10

*Cow 114.* The left front udder-quarter was infected with a streptococcus having beta hemolytic characteristics. No attempt was made to

further identify the organism. Milk from this quarter was alkaline in reaction at all times, and ranged in pH values from 7.10 to 7.67. Milk from the remaining quarters ranged in pH value from 6.54 to 6.78. The milk from the left front quarter, when inoculated with *S. lactis* and incubated for 24 hours showed pH values ranging from 6.44 to 7.30 as compared to pH values ranging from 4.41 to 5.23 for milk samples from the normal quarters held under similar conditions of incubation.

Microscopic examinations showed definitely that the milk from the left front quarter of this cow retarded the growth of *S. lactis*, thus confirming the findings as indicated by the study of the pH values.

*Cow 126.* Mastitis of a staphylococccic nature was present in the right front and left front quarters of this cow. Milk from the right front quarter ranged in pH value from 6.54 to 7.30 and milk from the left front quarter from 6.55 to 7.47. Milk from the two apparently normal quarters ranged from 6.49 to 6.78 in pH value. When inoculated with *S. lactis* and incubated for 24 hours the pH values of the samples from the right front quarter ranged from 4.54 to 7.05, for the left front quarter from 4.53 to 7.35 and from the two remaining and apparently normal quarters from 4.52 to 5.14.

The milk samples in which normal acid development did not occur had initial pH values of 6.9 and higher. Two samples, however, with pH values of 6.92 permitted *S. lactis* to develop in a normal manner as judged by decreased pH values.

*Cow 118.* The right front and right hind quarters of this cow showed definite evidence of mastitis due either to a staphylococcus or to an organism having the morphological characteristics of organisms of the genus *Corynebacterium* or to both, since these organisms were present in considerable numbers in both diseased quarters. The milk from these quarters ranged in initial pH values from 6.68 to 7.30 and from 6.66 to 7.05 respectively. The pH values recorded for the milk from the two remaining quarters varied from 6.49 to 6.82. After inoculation with *S. lactis* and incubating for 24 hours, milk from the right front quarter ranged in pH value from 4.68 to 7.10, from the right hind quarter from 5.70 to 7.04 and from the two apparently normal quarters from 4.30 to 5.10. Greater variation occurred with the milk from this cow in the initial pH value of the samples showing retarded acid development than occurred in the milk from cow 126. One sample with an initial pH value as low as 6.68 showed marked delayed acid development, whereas other samples with initial pH values up to 6.95 showed little or no delayed acid development.

*Effect of adjusting the pH value of mastitis milk to that of normal milk on the subsequent growth responses of S. lactis*

Since the most favorable reaction for the growth of *S. lactis* corresponds to that of normal milk, the relatively high pH values of mastitis milk tend

to create an unfavorable environmental condition for the best development of this organism. Also, a considerable amount of physiological activity is necessary on the part of the culture, in producing an amount of lactic acid sufficient to reduce the pH value of mastitis milk to that of normal milk. Numerous experiments, therefore, were carried out in which the pH value of the mastitis milk was adjusted to that of the normal milk from the healthy udder-quarters by the addition of lactic acid. Both the normal milk samples and the adjusted mastitis milk samples were inoculated with *S. lactis* and observations made in the usual manner.

On incubation the pH values of the adjusted mastitis milk, in almost every instance, failed to decrease as rapidly as did the pH values of the normal milk. Usually there was little or no appreciable change in the pH values of the adjusted mastitis milk during the first fifteen to twenty hours, after which time fairly rapid acid development took place with the final pH values dropping to approximately the same levels as those of the normal milk samples.

*Effect of mixing normal and mastitis milk on the subsequent growth of *S. lactis**

Numerous experiments were carried out in which varying proportions of normal and mastitis milk were mixed, inoculated with the test culture and frequent observations made during the incubating period.

The extent of the retarding effect depended on the degree of abnormality. Many of the samples of definitely abnormal milk in which the pH values were 7.2 and higher exerted a retarding effect upon the growth of *S. lactis* when mixed with normal milk in as little as 10 per cent concentrations. Other samples in which the growth of *S. lactis* was only slightly retarded, lost this characteristic when diluted with as little as 10 per cent of normal milk.

*Effect of pasteurizing mastitis milk on the subsequent growth responses of *S. lactis**

Since it has been shown by Hammer and Baker (8) that the growth of starter organisms is more rapid in milk that has been subjected to high pasteurizing temperatures, numerous experiments were carried out in which portions of mastitis milk were subjected to temperatures of 62.5, 65.5 and 68.5° C. for periods of 30 minutes. Pasteurizing at 62.5° C. had little or no effect on the subsequent growth of *S. lactis*. Pasteurizing some samples at 65.5–68.5° C. for 30 minutes tended to partially overcome the retarding influence on the growth of *S. lactis*. With most of the milk samples, however, heat treatment at these temperatures had little or no appreciable effect.

## DISCUSSION OF RESULTS

With the milks used in this study the retarding effects on the growth of *S. lactis* cannot be attributed to the high pH values nor to the presence of a thermolabile inhibiting substance such as that associated with excessive numbers of leucocytes. Although leucocyte counts were not recorded in this study, the samples that were definitely abnormal contained excessive numbers of these cells as indicated by the examination of stained preparations for the presence of *S. lactis*. In this respect the results reported herein differ from those of Whitehead and Cox (2) in which the inhibiting effect of abnormal milk containing large numbers of leucocytes was entirely removed by heating the milk for 30 minutes at 63° C. The results of this study tend to bear out the contention of Davis and McClemont (7) that the slow growth of *S. lactis* in mastitis milk probably is associated with the changed chemical composition of such milk.

While the extent of the retarding effect of mastitis milk usually was associated with the degree of abnormality such, however, was not always the case. Under the prevailing conditions of milk production it is unlikely that any considerable quantities of definitely physically abnormal milk would find its way into that used for commercial purposes. Certain occasions might arise, however, where milk, that appears normal in its physical properties yet possessing definite inhibiting action on the growth of *S. lactis*, may be present in sufficient amounts to interfere with manufacturing processes dependent upon the development of this organism.

The variations noted in the growth responses of *S. lactis* in the milks from the three cows used in this study may have been due in part, to the respective types of mastitis involved. Unfortunately, no milk for extensive study was available from a cow suffering with *Streptococcus agalactiae* type of mastitis, the type most common among dairy cows. However, in connection with this study, numerous observations were made using milk from *S. agalactiae* infected udder-quarters with the same general results.

## SUMMARY

The growth responses of *S. lactis*, as judged by changes in the pH values and microscopic examinations have been studied in separate samples of milk drawn from the infected and non-infected udder-quarters of cows suffering with mastitis.

Milk with an initial pH value greater than 6.9 usually failed to support the growth of *S. lactis* in an active manner, whereas normal milk from the other udder-quarters showed normal acid development. The growth responses of *S. lactis* varied somewhat in the milk drawn from the three cows under study.

Adjusting the pH value of mastitis milk to that of normal milk resulted in only partially overcoming delayed acid development. Mastitis milk treated in this manner changed little in pH value until after fifteen to twenty hours, after which time acid development was quite rapid.

The addition of as little as 10 per cent of very abnormal mastitis milk to normal milk had a retarding effect on *S. lactis* development.

Pasteurizing some samples of mastitis milk at 65.5° C.-68.5° C. for 30 minutes tended to partially overcome the retarding influence on the growth of *S. lactis*; however, with most of the samples studied heat treatment at these temperatures had little or no appreciable effect.

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## THE RELATIONSHIP OF MOISTURE IN SWISS CHEESE TO QUALITY AND YIELD

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The amount of moisture present is known to be important in the ripening of cheese. Sammis and Germain (11) have shown that, in Cheddar cheese, ripening is influenced by the ratio of moisture to solids-not-fat, and they pointed out that bacterial growth and chemical changes are more rapid in high-moisture cheese than in cheese that contains relatively less moisture. Van Slyke and Hart (13) showed that, in Cheddar cheese, an increase in the percentage of moisture "favors active chemical changes in the process of ripening," and attributed the increased ripening to the effect of moisture in diluting the products of fermentation and increasing the activity of bacteria and enzymes.

Excepting papers in Swiss journals describing work of Koestler (7), Dorner and Stähli (4), and Orla-Jensen (9), very little has been reported on the control of moisture in Emmentaler or Swiss cheese and the effect of moisture content on quality. Koestler showed that a finely-harped curd tends to retain moisture, and that the presence of cheese dust tends particularly to cause high moisture content, leading to excessive fermentation, oversetting, and generally defective ripening. Dorner and Stähli expressed the belief that an insufficient rate and amount of drainage is a common cause of defects, and that when high moisture content results in excessive final acidity, the low pH (high acidity) prevents the formation of normal eyes and causes the curd to be firm and "short" so that cracks appear instead of eyes during the ripening. A similar observation has been referred to in a previous publication (2) from these laboratories. Orla-Jensen found that the use of high cooking temperatures tends to dry the cheese curd in the kettle, but at the same time results in an inhibition of the ripening and a decrease in the rate of eye formation.

The following data on the percentage of moisture in normal Emmentaler cheese are taken from foreign literature. For green cheese: In 18 cheeses (averaging approximately 50 per cent fat in dry matter), average percentage of moisture, 37.08 (7); in 1 cheese, 43.99 (15); in 1 cheese, 40.92 (1); average of 20 green cheeses, 37.62 per cent moisture. For cured cheese:

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<sup>2</sup> The work on factory cheese described herein was conducted with the cooperation of the Departments of Dairy Industry of the University of Wisconsin and the Ohio State University.

In 4 cheeses, 34.71 (15); in 5 cheeses, 35.78 (1); in 4 cheeses, 36.05 (8); in 259 cheeses, 35.19 (12); in 14 cheeses, 35.34 (7); average of 286 cured cheeses, 35.21 per cent moisture. Winkler (16), in a discussion of this subject, stated that 33 to 35 per cent is a desirable range of moisture content, and that 38.2 per cent ought to be the upper limit. Orla-Jensen (9) has given values between 35 and 36.5 per cent as being representative for cured Emmentaler cheese.

Comparison of a large amount of data obtained in analyzing domestic Swiss cheese with the data cited above indicates that, even though Emmentaler cheese is cured longer than domestic Swiss cheese, much of our Swiss cheese is abnormally high in moisture content in comparison with Emmentaler cheese of similar age. There is a belief in some quarters, expressed particularly by cheese dealers, that the practice of incorporating excessive amounts of moisture in this and certain other varieties of cheese is detrimental to quality. On the other hand, it is commonly believed that the presence of a high percentage of moisture is accompanied by a proportionate increase in yield, although no data on this subject have been published.

The purpose of this paper is to present a report of information collected in a study of some of the factors causing variations in the amount of moisture in Swiss cheese and the effects of moisture content upon quality and yield.

#### METHODS

In the laboratory experiments, two cheeses were made daily from two lots of the same milk, under conditions and by methods as nearly as possible like those prevailing in the factories. The milk used in each kettle was weighed accurately in a receiving tank. Each cheese was weighed, and the percentage of moisture in the interior was determined, both when the cheese was one day old and when it was cut and graded. Both cheeses of each pair were made and cured in the same manner except for the experimental variation which was being studied. Plans were made to manufacture not less than 3 pairs of cheese under each experimental variation and, if the results were found to be inconsistent or if other important phases of the subject required more study, additional pairs were to be made so that averages for a larger number would be secured. Moisture data for green as well as cured laboratory cheese are presented, because it is believed that the former data are more representative of the effects of variables in the making process.

Laboratory cheeses were scored by an arbitrary, numerical scorecard system, which is intended to correspond as nearly as possible with the grading systems used by buyers and cheesemakers in the factories, and in which the relationship between grade and score is as follows: Fancy, 100 to 89; A or No. 1, 88 to 76; B or Special, 75 to 71; C or No. 2, 70 to 61; and D or Grinder, 60 or below. The principal points on the scorecard used, together

with the numerical value assigned to each, are as follows: eyes, 40; body and texture, 30; flavor, 20; and appearance, 10.

The data reported for factory cheese represent results obtained in the Bureau's portable laboratories, operating at factories in the States of Wisconsin and Ohio in cooperation with the Universities of these two States, and other results obtained in a factory in Pennsylvania. The study covers data on 226 cheeses made in 8 factories in Wisconsin, 160 cheeses made in 23 factories in Ohio, and 32 cheeses made in 1 factory in Pennsylvania—a total of 418 cheeses made in 32 factories. In the case of factory cheese the milk used in each cheese was not weighed and it was therefor not possible to obtain accurate figures for yield; neither was it feasible to obtain samples for analyses except at the time the cured cheeses were graded and sold.

#### RESULTS

Statistical averages of data for 218 laboratory Swiss cheeses are presented in table 1, showing the relation of percentage of moisture in green cheese to

TABLE 1

*Data showing average score and yield of experimental Swiss cheese of different moisture content (218 60-lb. cheeses cured 2-1/2 to 3 months)*

Moisture in green cheese	Number of cheeses	Average total score	Average eye score	Pounds cheese per cwt. milk		Shrinkage in curing
				Green	Cured	
<i>per cent</i>						
Above 39.7	15	65.4	16.8	8.98	8.14	9.35
39.1-39.7	27	74.8	24.0	8.80	7.98	9.32
38.5-39.1	63	74.6	23.7	8.85	8.04	9.15
37.9-38.5	80	74.0	22.8	8.82	8.04	8.84
Below 37.9	33	73.4	22.3	8.89	8.12	8.66

total score, eye score, yield of cheese per hundred pounds of milk, and shrinkage of cheese in curing. Each cheese weighed between 56 and 61 pounds when made. Data in which the experiments involved abnormal variations are not included in the tabulations in table 1,—i.e., cheese made from unclarified milk (table 3, variation No. 10), cheese made from milk which had been ripened with lactic starter (variation No. 6), and cheese made without the use of streptococcus starter (variation No. 1) are excluded.

The data indicate that a percentage of moisture above 39.7 in the green cheese is usually detrimental to the quality of the cheese. This detrimental effect was most evident in the eye formation. The defect known as "oversetting," a condition in which the eyes are too numerous and too small, was very pronounced in most of the high-moisture cheeses, and they usually tended to rise more rapidly and to a greater extent than low-moisture cheeses. Along with poor eye formation there often occurred, in high-moisture cheeses, a defective flavor usually characterized as "unclean."

Relationships between actual and theoretical yields, both for green and cured cheese, are shown in figure 1. The theoretical yields shown in line

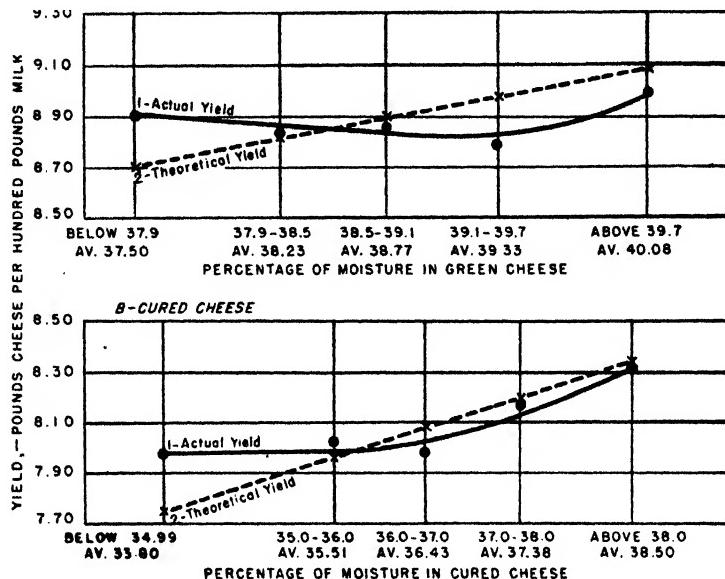


FIG. 1. Data showing average yield of experimental Swiss cheese of different moisture content (218 60-lb. cheeses).

2 are the yields that would be expected if the average yield of cheese in each moisture group was strictly proportional to the percentage of moisture. [The theoretical yield values were calculated by multiplying the average yield of cheese (green, 8.86 pounds; cured, 8.04 pounds) by the average percentage of dry matter (green, 61.45 per cent; cured, 63.81 per cent), and dividing the result by the percentage of dry matter in each respective moisture group.] A comparison of theoretical yields with actual yields shows that as the percentage of moisture in the cheese increased, the increase in yield was not proportional to the increase in percentage of moisture; as the percentage of moisture decreased, the actual yield was greater than the theoretical yield.

The data were therefore tabulated to determine the relationships between percentage of fat in kettle milk, percentage of moisture in cheese, and yield of cheese. The results shown in figure 2 indicate that as the percentage of fat in the kettle milk increased, the percentage of moisture in the green cheese tended to decrease. This fact has been pointed out previously by European workers (3, 9, 12, 16). It is indicated also in figure 2 that as the percentage of fat in the kettle milk increased, the yield of cheese tended to increase in spite of the decrease in moisture content.

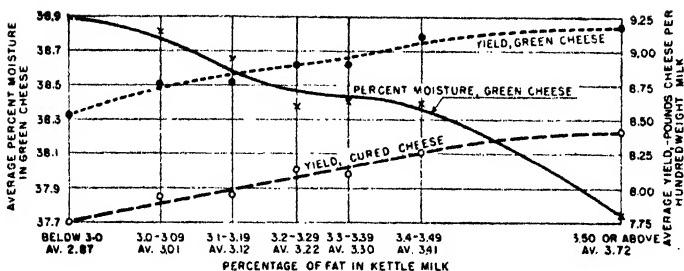


FIG. 2. Data showing average percentage of moisture and average yield of experimental Swiss cheese made from milk of different fat content (218 60-lb. cheeses).

Tabulation of data for 45 laboratory cheeses made from milks containing the same percentage of fat (3.1 per cent) indicated that, when the factor of fat variations in milk is eliminated, there is some increase in yield of cheese as moisture content increases, but the increase in yield is not proportional to the increase in moisture content. Cheeses in this group containing less than 35.0 per cent moisture when cured had an average cured yield of 7.92 pounds; those containing more than 38.0 per cent moisture had an average cured yield of 8.33 pounds.

While it is possible that the above results are not strictly typical of yields that may be expected to occur in factory cheese, for the reason that losses in moisture and in yield are smaller per unit of weight in the larger wheels, it is believed that the trends in the two cases are similar and that variations in the milk or making process will produce similar effects in the yield of green cheese in either case. Effects of manufacturing variations upon yield will be referred to below.

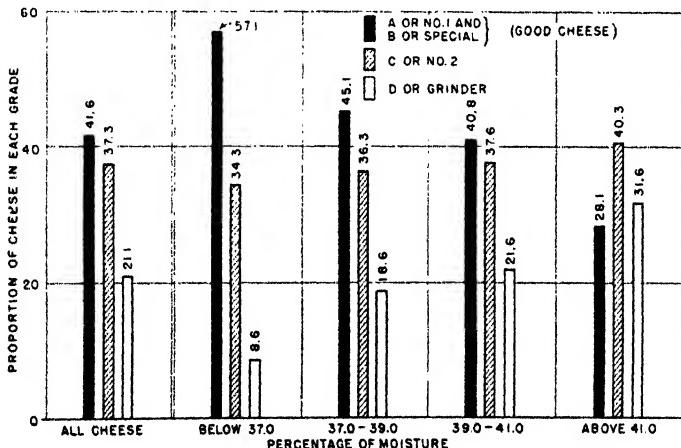


FIG. 3. Relation of moisture content to quality of Swiss cheese (418 factory cheeses cured approximately 2½ months).

Averages of data on moisture and quality for 418 factory cheeses are shown in figure 3. These data tend to confirm the laboratory results. The proportion of cheese of the A and B grades (good cheese) was relatively high in those cheeses which contained less than 37 per cent moisture—a figure which corresponds best with figures quoted above for foreign Swiss or Emmentaler cheese. Similarly, the proportion of cheese of the grinder grade (poor cheese) increased progressively as the average percentage of moisture increased from below 37 to above 41 per cent.

The average percentage of moisture in the 418 factory cheeses was 39.40. Averages of moisture data for the cheeses in each of the four grades were as follows: A, 55 cheeses, 38.70 per cent; B, 119 cheeses, 39.30 per cent; C, 156 cheeses, 39.46 per cent; and D, 88 cheeses, 39.88 per cent. These figures show a consistent downward trend in quality as moisture content increased.

Average moisture figures and grades were also tabulated for each factory. These were divided into two groups, as follows: (a) 8 factories in each of which the average percentage of moisture in the cheeses sampled was greater than 39.4 per cent, and (b) 24 factories in each of which the average percentage of moisture in the cheeses sampled was less than 39.4 per cent. Of 226 cheeses sampled in the first group (moisture above average), 31.0 per cent were graded No. 1 or special, corresponding to A or B grade (good cheese); 39.4 per cent were graded No. 2, corresponding to C grade; and 29.6 per cent were graded grinder, corresponding to D grade. Of 192 cheeses sampled in the second group (moisture below average), 54.2 per cent were graded No. 1 or special (good cheese); 34.9 per cent were graded No. 2; and 10.9 per cent were graded grinder. The greater proportion of good cheese was produced in those factories in which cheese of relatively low moisture content was manufactured.

The possibility was considered as to whether the effect of poor quality of milk might have predominated in producing poor average quality in high-moisture cheese. Tabulations of the data for factory cheese showed, however, that the average methylene blue reduction time of the milks used in the cheeses in the low-moisture groups shown in figure 3 was actually slightly shorter than that of the milks used in cheeses in the high-moisture groups. The data indicated that the use of milk having a short methylene blue reduction time resulted in a general but slight tendency toward a decrease in moisture content in the cheese; this is probably the result of the curd-drying effect of overripe milk, resulting from a rapid production of acidity in the kettle and on the press when such milk was used. However, it has been shown by Rogers, Hardell and Feutz (10), and also by Erekson and his coworkers (5), that a short reduction time usually results in a rather markedly detrimental effect upon the average quality of cheese. They found that the proportion of good cheese was considerably greater when the reduction time was more than 3 hours than when it was less.

The results for factory cheese were tabulated on the basis of a combination of methylene blue reduction time and moisture content of cheese. Resulting data are illustrated in figure 4. It is shown that a methylene

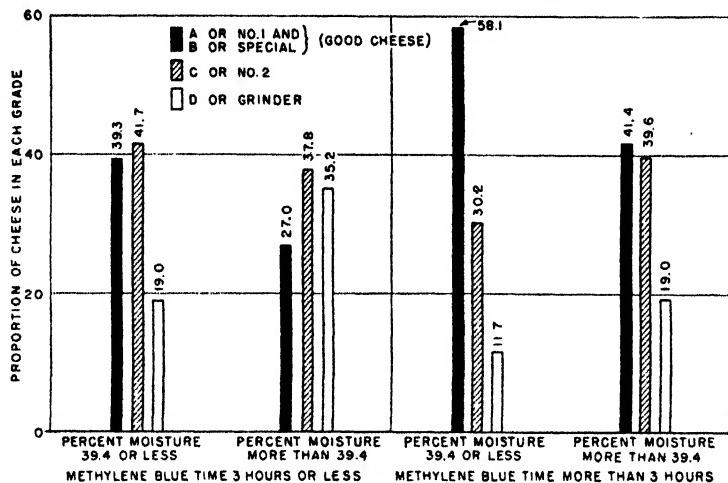


Fig. 4. Relation of methylene blue reduction time of milk and percentage of moisture in cheese to quality of Swiss cheese (418 factory cheeses).

blue reduction time of more than 3 hours together with a relatively low percentage of moisture produced the greatest proportion of good cheese, while a reduction time of less than 3 hours together with a relatively high percentage of moisture in the cheese produced the greatest proportion of grinders or cheese of poor quality.

Our data did not show that the factors that cause an increase in moisture content always result in impairment of quality of cheese. They did indicate that the chances of making a relatively large proportion of good cheese are impaired when the moisture content is excessively high. Other factors, in addition to those considered in this paper, unquestionably play a part in influencing quality.

It will be noted that the range of values for moisture in laboratory green cheese shown in table 1 is lower than the range in factory cured cheese in figure 3. We have found it difficult, when using normal methods, to incorporate as large a percentage of moisture in laboratory cheese as is often present in factory cheese. Analyses show that one reason for this difference is the fact that smaller cheeses lose moisture somewhat more rapidly than larger ones by drainage on the press. A larger proportion of the milk used in certain cheese-producing areas has a relatively lower fat and total solids content and a softer curd—factors which tend to produce an increase in moisture content. Moreover, as has been demonstrated (13) in the case of the

Cheddar variety, smaller cheeses lose a greater percentage of moisture than larger ones by evaporation during curing.

Before results of variations in the making process are discussed, it seems desirable to present data showing temperature conditions and the amount and rate of moisture loss from the curd during this process. Such data, taken from work on Emmentaler cheese reported by Koestler (7) and also from our results on laboratory Swiss cheese, are shown in table 2. The lab-

TABLE 2  
*Data showing temperature conditions and losses of moisture during Swiss cheese manufacturing process*

Stage in manufac-turing process	Data by Koestler, average of 4 cheeses			Data by this laboratory, average of 30 cheeses			
	Temp.	Mois-ture	Loss of moisture	Time elapsed	Temp.	Mois-ture	Loss of moisture
Before curdling .....	31.75	88.4	0.0	0	32.5	87.8	0.0
Beginning of heating .....	80.75	63.3	25.1	77	32.0	64.9	22.9
End of heating .....	53.0	54.8	8.5	31	53.0	53.6	11.3
Dipping .....	50.3	51.8	3.0	48	50.5	52.6	1.0
3 hrs. after dipping .....	...	...	...	...	48.3	42.1	10.5
21 hrs. after dipping .....	...	37.0	14.8	...	39.0	39.1	3.0

oratory data show percentages of moisture in the uncoagulated kettle milks, in samples of curd taken from the kettle with a strainer and allowed to drain for 2 minutes before being analyzed, and in plug samples taken from the interiors of the cheeses. It is shown that the greatest losses of moisture from the curd occur during the time when rennet action is most rapid after curdling, and again when the curd is dipped and placed on the press. The greatest proportion of whey loss from the cheese after dipping occurs very early on the press, at a time when the activity of the streptococci is relatively great in comparison with that of the lactobacilli.

There is presented in table 3 a list of those factors which in our experiments were found to influence the percentage of moisture in experimental Swiss cheese and the resulting average yields. Only a few of the apparently more important effects of the variations shown will be discussed.

In variation No. 1, all cheeses made without streptococcus starters were grinders, and their yields and moisture contents were comparatively high.

Results shown in variation No. 3 confirm the work of Koestler (7), who found that fine harping caused a considerable increase in moisture content. Even though fine particles contained a smaller percentage of moisture (and fat) than large ones, cheese moisture content was greater because of more retention of moisture on the greatly increased surface area of fine particles, and because fine particles tend to cause a stopping-up or clogging of drainage capillaries. In our experiments, the finely-harped cheeses were not in-

TABLE 3

*Data showing effects of variations in the making process upon the average moisture content and yield of experimental Swiss cheese\**

Variation in making process	Number of pairs	Moisture in green cheese		Yield cured cheese per cwt. milk	
		Ave.	Diff.	Ave.	Diff.
		%	%	lb.	lb.
1. No streptococcus starter . . . . .	3	40.16		8.39	
25 cc. streptococcus starter† . . . . .		38.46	-1.70	8.16	-0.23
2. Holstein milk standardized to 3.5% fat . . . . .	3	38.80		7.94	
Jersey milk standardized to 3.5% fat . . . . .		37.50	-1.30	8.25	+0.31
3. Harped fine . . . . .	5	38.97		7.85	
Harped coarse . . . . .		37.94	-1.03	7.96	+0.11
4. 10% water added to kettle milk . . . . .	5	39.49		7.74	
No water added . . . . .		38.47	-1.02	7.95	+0.21
5. Normal milk standardized to 2.9% fat . . . . .	30	38.68		7.68	
Normal milk standardized to 3.4% fat . . . . .		37.69	-0.99	8.23	+0.55
6. Milk not ripened . . . . .	5	38.40			
Milk ripened with lactic starter . . . . .		37.50	-0.90		
7. 0.03% sodium citrate added to milk . . . . .	5	40.08		7.78	
No citrate added . . . . .		39.22	-0.86	7.92	+0.14
8. Heated in 26 minutes . . . . .	10	38.73		8.14	
Heated in 60 minutes . . . . .		37.91	-0.82	8.03	-0.11
9. 9 cc. streptococcus starter† . . . . .	4	39.37		7.91	
27 cc. streptococcus starter† . . . . .		38.87	-0.50	7.85	-0.06
10. Milk not clarified . . . . .	18	38.70			
Milk clarified . . . . .		38.20	-0.50		
11. 5 cc. rennet† . . . . .	3	38.43		8.14	
10 cc. rennet† . . . . .		38.00	-0.43	8.12	-0.02
12. Set at 31° C. . . . .	3	38.63		7.70	
Set at 35° C. . . . .		38.20	-0.43	7.66	-0.04
13. 20 cc. lactobacillus starter } † . . . . .	6	38.87		8.15	
12.5 cc. streptococcus starter } . . . . .					
60 cc. lactobacillus starter } † . . . . .		38.49	-0.38	8.19	+0.04
40 cc. streptococcus starter } . . . . .					
14. Cooked 50.5° C.; stirred 16 min. . . . .	6	38.61		8.25	
Cooked 54° C.; stirred 60 min. . . . .		38.24	-0.37	8.12	-0.13
15. 5% cold water added before dipping . . . . .	4	38.90		8.20	
No water added . . . . .		38.65	-0.35	8.29	+0.09
16. Cut 28 min. after setting . . . . .	3	38.93		7.96	
Cut 42 min. after setting . . . . .		38.59	-0.34	7.90	-0.06
17. Combination of above variations‡ . . . . .	6	40.50		8.47	
Wet . . . . .		37.53	-2.97	8.43	-0.04

\* The following variations resulted in average difference of less than 0.25 per cent moisture:

Foreworking 60 min. compared with 20 min.; yield 8.13 compared with 8.25.

Cooking to 54.5° C. compared with 50.5° C.; yield 8.05 compared with 8.07.

Stirring out 50 min. compared with 15 min.; yield same.

Heavy pressing compared with light pressing; yield 8.11 compared with 8.08.

† Per cwt. milk.

‡ Conditions combined included variations Nos. 3, 8, 9, 11, 12, and 14.



FIG. 5. Low-moisture and high-moisture cheeses made from separate portions of the same milk: 112—1, 37.51% moisture (green); score, 74 (cured)—112, 40.55% moisture (green); score, 50 (cured).

ferior in quality. The yield of cheese, however, was reduced 0.11 pound by fine harping. The wheys from the 5 finely-harped cheeses contained an average of 0.64 per cent fat and 6.80 per cent total solids; corresponding figures for the coarsely-harped ones were 0.52 and 6.65 per cent.

Effects of variations in fat content of milk, referred to earlier in this paper, are pointed out in variation No. 5. Milk standardized to an average of 2.9 per cent fat yielded green cheese containing an average of 38.68 per cent moisture and 44.4 per cent fat in dry matter, and portions of the same milk standardized to 3.4 per cent fat yielded cheese containing an average of 37.69 per cent moisture and 49.1 per cent fat in dry matter. The high-fat cheeses, which contained the least moisture, were generally superior in quality, especially with respect to texture and flavor. The average yield of cured cheese of the low-fat group was 7.68 pounds per hundred pounds of milk and that of the high-fat group was 8.23 pounds. Corresponding yields of cured cheese per pound of fat in milk were 2.66 and 2.34 pounds, respectively. Methods of standardization, and effects of percentage of fat on quality, will be discussed in a later paper.

The data shown under variations No. 8, 14, and 16 indicate that expulsion of moisture is favored by extending the time of certain stages in the making process. Our results indicate that this is particularly true when acidity is developing rapidly in the kettle contents. Under this condition the drying effect of a slow, prolonged heating period is especially marked. Vas (14) has shown that long-continued heating not only increases the specific gravity and decreases the water-holding capacity and surface adhesiveness of the granules, but also stiffens the granular structure and promotes the whey-carrying effectiveness of the capillaries through which drainage occurs after dipping.

Long foreworking resulted in a slight decrease in yield, but had little effect in decreasing moisture content except when acid was being formed rapidly in the kettle.

In variations Nos. 1, 9, and 13, pH values at dipping and at 3 hours later showed relatively rapid development of acidity in the cheeses containing the larger amounts of starter; the relatively lower moisture content in these cheeses demonstrates the effect of acidity in drying the curd. Numerous other experiments have demonstrated conclusively that acid development is an important factor in drying the cheese in the kettle and on the press. The expulsion of moisture in the presence of acid is readily explained by the fact that, as the reaction of the curd changes toward pH 4.6, which is the isoelectric point and the point of least solubility of casein, the curd shrinks and tends to lose its ability to combine with or hold water. It has been shown (2), however, that when acidity develops rapidly near the rind and slowly in the interior, the high-acid rind forms a barrier that hinders proper drainage of the cheese on the press. Such a condition may exist when the number or activity of streptococcus starter organisms, which grow relatively early in the interior of the cheese, is low in comparison with that of the lactobacilli. Our results indicate that an increase in the amount or activity of the streptococcus starter has a somewhat greater effect in promoting drainage than an increase in the amount or activity of the lactobacillus starter.

In variation No. 6, cheese made from milk which had previously been ripened slightly with a lactic starter containing only *Streptococcus lactis* organisms showed a relatively rapid development of acidity on the press after dipping, even though the activity of these organisms has been shown to be stopped by the temperature used in the cooking process (6). These ripened-milk cheeses in which acid was produced rapidly contained some glass (glaesler defect) and were slightly short and inelastic in texture and inferior in quality.

Dorner and Stähli (4) found that when water was added to the kettle contents just previous to dipping (variation No. 4), the resulting dilution of the whey caused a decrease in the amount of lactose contained in the cheese and hence a decrease in the ultimate amount of acid which was formed in the one-day-old cheese. The higher pH value (lower acidity) of the one-day-old cheese resulted in an increase in eye formation. These observations are confirmed by our results—the addition of water caused an average decrease in final acidity (increase in pH value) in the one-day-old cheese, and the tendency toward oversetting was greatest in those cheeses having the highest average pH values when one day old.

Orla-Jensen (9) found that increasing the cooking temperature from 48° to 56° C. caused the percentage of moisture in the cured cheese to decrease from 36.64 to 35.33 per cent, and he quoted earlier work of Schaffer which

showed a similar drying effect resulting from high cooking. Both of these workers found that the use of high cooking temperatures caused a decrease in the rate of protein decomposition and ripening in the cheese. Orla-Jensen believed that the cooking temperature should be high enough to aid in drying the cheese curd sufficiently without the necessity of a long stirring-out period, but not sufficiently high to seriously inhibit ripening and the formation of eyes. He believed that the use of a relatively high cooking temperature together with an addition of eye-forming organisms would increase the possibility of making cheese of relatively high quality. Our laboratory results, in general, confirm the latter observation.

In our experiments, an increase of 3° C. in cooking temperature caused an average decrease of less than 0.25 per cent in moisture in the green cheese. Similarly, the decrease was very small when the duration of the stirring-out period was increased markedly. A combination of high cooking temperature with long stirring-out time (variation No. 14), not ordinarily used in factory practice, resulted in a decrease of 0.37 per cent moisture, and resulted in a slight improvement in the average quality of the experimental cheeses.

High cooking temperatures regularly caused the curd to be relatively dry at the end of the cooking and of the stirring-out period. However, high cooking temperatures retarded acid formation on the press rather markedly, and the high-cooked cheeses lost moisture comparatively slowly after dipping, presumably because of slow pH change. When the kettle contents were stirred out for relatively long periods, it was found that cooling was more rapid in the kettle than on the press. Long stirring-out periods resulted in low dipping temperatures which served to accelerate, to a slight extent, both acid formation and drainage on the press.

Analytical results showed that the effects of the variations listed in table 3 were not strictly cumulative. In securing the data shown in variation No. 17, six pairs of cheese were made in which the following factors were combined to produce high moisture content in one cheese of each pair: less rennet, less streptococcus starter, lower setting temperature, finer harping, lower cooking temperature, and a shortening of each stage of the making process; in addition, in two cheeses out of the six, the percentage of fat in the kettle milk was reduced to 2.9 as compared with 3.5. By combining these factors against an opposite condition in each case, it was found that the percentage of moisture could be varied within a range of about 3 per cent. This range evidently would differ with conditions involving principally the properties of the milk and the size of the cheese.

In figure 5 is shown a photograph of a pair of laboratory cheeses in which the lower cheese contained a relatively high percentage of moisture and shows oversetting and irregular eye formation. These cheeses were made in experiments described under variation No. 17, table 3.

It has been pointed out above that a relatively low percentage of fat in kettle milk resulted in a relatively high percentage of moisture but a low yield in cheese, and that fine harping had a similar effect. Other variations in which relatively high moisture content was accompanied by a decrease in yield were: No. 2, the use of Holstein or low-solids milk; No. 4, the addition of water to milk; No. 7, the addition of sodium citrate, which produced soft-curd milk; No. 13, the use of relatively small amounts of starters; and No. 15, the addition of water before dipping.

Studies of the analytical and yield data obtained in the present work indicated that, in addition to the physical effect of the amount of moisture present, the following factors tend to influence yield:

1. The use of milk standardized to a relatively low fat content results in relatively high moisture content in the cheese but decreases the yield because of the decrease in amount of fat in the cheese.
2. Milk low in solids-not-fat content tends to have a low curd tension; the use of such milk results in relatively high moisture content in cheese but decreases the yield because of low solids content and because of an increase in the proportion of solids lost in the whey.
3. High-moisture cheese loses a relatively large proportion of its moisture, and of its yield, during curing.
4. Some of the procedures used to increase moisture content cause losses in yield by causing an increased loss of solids in the whey.

#### SUMMARY

Correlations of analytical data with grades of 218 experimental and 418 factory Swiss cheeses indicated that the presence of an excessive amount of moisture is generally detrimental to the quality of the cheese.

Laboratory results on yields of cheese per hundred pounds of milk indicated that some of the manufacturing variations which may be used to bring about the inclusion of excess moisture actually result in decreases in yield, and that in general the inclusion of a comparatively large amount of moisture does not result in sufficient increase in yield to justify the practice.

A study is presented showing effects of numerous variables in the milk and making process upon the moisture content of the cheese.

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A STUDY OF THE CHARACTERISTICS OF A MILK SUPPLY  
AS RELATED TO THE MANUFACTURE OF PLAIN  
CONDENSED SKIMMILK FOR ICE  
CREAM MAKING<sup>1</sup>

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It is often economically desirable for dairy manufacturing plants to be self-sufficient in supplying the milk ingredients of their products. In the manufacturing of ice cream a concentrated milk is used as a source of added serum solids to improve the body and texture. Since the production of milk fluctuates during the seasons it would be desirable, perhaps, to concentrate the solids of the surplus milk to be used during the period of reduced production.

It was in an effort to store these milk solids with a minimum of processing that led to the freezing and storing of condensed skimmilk by some ice cream manufacturers. In many instances this practice of condensing the surplus skimmilk, freezing and holding it frozen until used to make the ice cream mix, has been carried out successfully; yet in other cases it has proved to be unsatisfactory because after the skimmilk had thawed it was found that precipitation of the protein had occurred.

Therefore, if it were possible to determine by some chemical or physical test the degree of stability of the protein fraction of the milk supply, the suitability of the milk to being stored frozen in the form of condensed skimmilk could be determined.

STATEMENT OF PROBLEM

The purpose of the investigation was to study the characteristics of a milk supply which appears to be associated with the ability of the protein fraction to remain stable after the skimmilk obtained by centrifugal separation had been condensed and then stored frozen. The study was conducted by making chemical and physical tests on representative samples of milk taken at specific intervals in the manufacture and storage of the plain condensed skimmilk. Physical and chemical tests were also used to determine the effect of a change in the characteristics of condensed skimmilk during storage upon an ice cream mix and the resultant ice cream.

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<sup>2</sup> The data presented in this paper are from a study made by the junior author under the supervision of the senior author in partial fulfillment of the work required for the degree of Master of Science.

## REVIEW OF LITERATURE

Reichart and Corley (8) have presented a review of the work done previous to 1938. In their work they found that after storing plain condensed skimmilk at  $-17.8^{\circ}$  C. ( $0^{\circ}$  F.) for three months, the condensed skimmilk was coarser than when first made, and at the fourth month a partial gel had been formed. From the fifth month on the milk had formed a complete gel and in addition, at the eighth and ninth months some wheyng off was observed on thawing. However, they found that in spite of the poor appearance of the frozen condensed skimmilk after being melted, the appearance of the final ice cream mix was not affected and they had no trouble in processing it. They recommended, however, that the skimmilk should not be stored over six months.

Openlander and Erb (6) reported that condensed skimmilk if properly frozen and stored could be used as a satisfactory source of serum solids. They used a storage period of 13 weeks and found that the first noticeable defect which might occur in the ice cream made from frozen condensed skimmilk was the curdled appearance on melting.

Winn (13) found that plain condensed skimmilk, as well as sweetened condensed skimmilk, could be frozen and stored at  $-17.8^{\circ}$  C. ( $0^{\circ}$  F.) for periods up to 3 months without any detrimental effect upon their physical appearance. He found, however, that superheated condensed skimmilk showed signs of protein precipitation at the end of one month of storage, and the precipitation increased rapidly as the storage period continued up to 3 months.

## EXPERIMENTAL METHODS

The whole milk supply used in this study came from an area adjoining Lincoln, Nebraska. After being examined to make sure it had no objectionable off-flavor, the milk was pasteurized at  $145^{\circ}$  F. for 30 minutes; then cooled to a temperature of  $90$ – $100^{\circ}$  F. before being separated. Immediately after separation the skimmilk was preheated in 150-gallon lots to  $150^{\circ}$  F. It was then drawn into a Roger 26-inch vacuum pan (stainless steel) where it was condensed under a vacuum of about 25 inches, until the total solids content as determined by the Baume hydrometer was approximately 33.5 per cent.

Between 50 and 52 pounds of the condensed skimmilk were placed in new 5-gallon tinned lard cans and stored at  $-17.8^{\circ}$  C. ( $0^{\circ}$  F.). Altogether, 9 lots of whole milk were processed at approximate intervals of 4 weeks from October through May.

A 300-pound ice cream mix was made from each lot of condensed skimmilk when fresh and again after being stored frozen for four weeks. The frozen condensed skimmilk was held in water at  $21.1^{\circ}$  C. ( $70^{\circ}$  F.) over night to allow it to melt before it was incorporated in the ice cream mix. The mix

was calculated to contain 14 per cent milk fat, 10 per cent serum solids, 15 per cent sugar, and 0.25 per cent gelatin. In each case the cream and skim-milk required to furnish the milk fat and serum solids not furnished by the condensed skimmilk was not more than 24 hours of age. After being held at 71.1° C. (160° F.) for 20 minutes the mix was homogenized with a two-stage homogenizer (Manton-Gaulin) at 3000-pound pressure with 500-pound on the second stage.

After aging 24 hours at 5 to 6° C. (41 to 43° F.) the ice cream mix was frozen in a horizontal, direct-expansion 40-quart freezer. Three batches of mix were frozen, the first one being a preliminary batch. The amount of mix used in the preliminary trial was 45 pounds, while in the following two runs the amount was 42 pounds. The temperature of the ice cream mix at the beginning of the freezing process, the time required to lower the temperature of the contents of the freezer to -4.4° C. (24° F.) and the temperature of the refrigerant when this temperature of -4.4° C. (24° F.) was reached were recorded.

Overrun readings were taken by means of the Mojonnier overrun tester immediately after the refrigerant was shut off and at minute intervals thereafter until the maximum overrun had been obtained.

*Proceedings of sampling.* Quart samples of the whole milk were obtained before and after pasteurization of the skimmilk before preheating, and of the condensed skimmilk while fresh and after being stored frozen 4 weeks. A sample of each ice cream mix was taken at the time of freezing and a sample of ice cream was taken in quart sealright containers directly from the freezer at 100 per cent overrun and held in the hardening room at -17.8° C. (0° F.). The other samples were held in an ice and water bath.

*Analytical procedure.* The raw whole milk was analyzed to determine whether or not it was normal in chemical composition. Milk fat and total solids were determined by the Mojonnier method (4). The lactose, total protein, casein, and ash were determined according to the methods outlined in A.O.A.C. (5). A modification of the method of Rosswell (10) was used to determine the chloride content. Forty ml. of distilled water were added to 10 grams of milk in an Erlenmeyer flask and, after thorough mixing, the contents were titrated with  $\text{AgNO}_3$  of such a concentration that 1 ml. equalled 1 milligram of chlorine in 10 grams of the sample. One ml. of a 10 per cent solution of potassium chromate was added to the flask as an indicator.

The pH of the samples of whole milk and skimmilk was determined by means of the quinhydrone electrode using a type K Leeds and Northrup potentiometer at 25° C. (77° F.).

The titratable acidity was determined by titrating 25 grams of the sample which had been diluted with 25 ml. of  $\text{CO}_2$  free double distilled  $\text{H}_2\text{O}$ , with 0.1 normal  $\text{NaOH}$  using 10 drops of a 1 per cent solution of phenolphthalein.

The alcohol test of Dahle and Peyenson (1) was used to indicate protein stability and the least amount of 95 per cent ethyl alcohol required to bring about the first noticeable indication of coagulation in a 5 ml. sample was designated as the alcohol number. In all cases water was added prior to the alcohol so that the total volume of the addition (water plus alcohol) amounted to 10 ml. Also, the phosphate test of Ramsdell, Johnson and Evans (7) was used. However, when applied to condensed skimmilk the number of seconds submersion in the boiling water required to bring about indications of coagulation were recorded.

The milk fat and total solid content of the ice cream mixes were determined in duplicate by the Mojonnier method. The titratable acidity and pH were determined as they were in the case of the samples of milk. A heat coagulation test based on a modification of the method of Howat and Wright (3) was used. Three ml. of the ice cream mix were pipetted into a 15 × 125 mm. Pyrex test tube which was then closed with a rubber stopper. The stoppered test tube was then immersed in an oil bath maintained at 110° C. ± 0.5° C. (230° F.) until visible coagulation occurred; the time in minutes required to bring this about was recorded. The viscosity of the ice cream mix was determined by means of a Gramercy Model MacMichael Viscometer operated at 20 r.p.m. using a No. 30 wire and a 100 ml. sample at 5° C. (41° F.).

Approximately 10 days after freezing, the quart ice cream samples were removed from the hardening room and cut into two equal portions of one pint each. One pint was scored for body and flavor. The second pint was set upon a  $\frac{1}{4}$ -inch mesh wire, resting upon the rim of a heavy 5-inch funnel the end of which led into a mouth of a 100 ml. graduated cylinder which had been previously tared. The number of minutes required for the first drop, first 50 ml. and the first 100 ml. of melt to collect were recorded as was the weight of the first 100 ml. of the melt. The sample of ice cream was allowed to melt at room temperature, in order to simulate actual practical conditions as much as possible.

#### EXPERIMENTAL RESULTS

*Effect of processing upon the pH, titratable acidity, alcohol number and phosphate test.* Chemical analysis showed that all nine lots of milk used were normal in regard to the amount present of each of the constituents determined. The pH of the raw whole milk did not vary significantly from the usual range of 6.4 to 6.8.

As shown in table 1 it was found that pasteurization increased the pH, decreased the titratable acidity and tended to increase the alcohol number. The titratable acidity values of the whole milk ranged from .150 to .177 per cent calculated as lactic acid. It was found that the titratable acidity of the raw whole milk decreased from October through February, then from March through May it tended to increase again. The pH of the skimmilk

TABLE 1

*Effect of pasteurization on the pH, titratable acidity and alcohol number of whole milk*

Date pro- cessed	Tests used					
	Before pasteurization			After pasteurization		
	pH	Titratable acidity	Alcohol number	pH	Titratable acidity	Alcohol number
10-21-38	6.58	.18	5.0	6.60	.17	6.0
11-18-38	6.59	.17	6.5	6.61	.16	7.0
12-28-38	6.59	.16	8.0	6.63	.14	8.0
1-27-39	6.54	.16	7.0	6.57	.15	8.0
2-24-39	6.68	.15	8.5	6.70	.15	8.5
3-24-39	6.70	.17	8.5	6.71	.16	8.5
4-14-39	6.73	.16	8.5	6.70	.15	9.0
5-5-39	6.50	.17	8.0	6.60	.17	8.2
5-26-39	6.37	.17	8.3	6.48	.16	8.5

was decreased noticeably by condensing. Before condensing, the range in pH for the skimmilk was from 6.64 to 6.69; after condensing, the range was from 6.04 to 6.33. There was no apparent seasonal trend in the pH of either product. The titratable acidity of the skimmilk was greatly increased by condensing. The increase was proportional to the increase in solids content. All samples were normal and there was some indication that the percent acidity found in the fresh skimmilk followed the same trend as it did in the raw whole milk.

The skimmilk had a higher alcohol number before condensing than the original milk supply, but a lower alcohol number after being condensed due to the influence of the total solids of the products. The range in alcohol numbers was not great, being from 8.5 to 9.5 for the skimmilk and 4.0 to 5.0 for the condensed skimmilk but with no definite seasonal trend shown.

In the case of the phosphate test it was found that (with one exception) the same fluctuations of protein stability were found in the condensed skimmilk as was indicated by the alcohol number. This one exception was in the lot of condensed skimmilk processed April 14, 1939. In this instance the protein was the most stable of any lot of condensed skimmilk studied, as was shown by the phosphate coagulation time of 70 seconds, yet this increased stability over the average of 55 seconds was not shown by the alcohol number of 4.0 which was lower than some of the other lots of condensed skimmilk prepared in this study.

*Effect of freezing and storing upon the character of the condensed skimmilk.* No evidence of wheyng off could be seen at any time in the frozen condensed skimmilk after being stored at  $-17.8^{\circ}$  C. ( $0^{\circ}$  F.) for 4 weeks. Other than being slightly sandy, the frozen condensed skimmilk melted down to yield a product similar to the fresh condensed skimmilk in body and texture.

Freezing and storing of the condensed skimmilk for 4 weeks caused an increase in titratable acidity and with one exception a decrease in the pH.

With one exception the protein stability of the condensed skimmilk as measured by the alcohol test was affected only slightly by storing frozen for 4 weeks. The data found are shown in table 2. In the remaining 8 trials the

TABLE 2

*The effect of storing plain condensed skimmilk at -17.8° C. (0° F.) on certain properties*

Date condensed	Total solids	Length of storage period	Test used			
			pH	Titratable acidity	Alcohol number	Phosphate test coagulation time
October 21, 1938 .....	33.97	0	6.148	.833	4.5	50
	33.97	4	5.978	.867	2.5	50
November 18, 1938 ..	35.20	0	6.203	.732	4.5	50
	35.20	4	6.086	.754	4.5	50
December 28, 1938 .....	35.58	0	6.104	.418	5.0	60
	35.58	4	6.071	.572	4.5	50
January 27, 1939 .....	33.20	0	6.038	.637	4.5	55
	33.20	4	6.198	.675	4.5	50
February 27, 1939 .....	32.95	0	6.332	.641	4.0	55
	32.95	4	6.285	.673	3.8	50
March 24, 1939 .....	32.13	0	6.278	.583	4.5	60
	32.13	4	6.218	.677	4.5	60
April 14, 1939 .....	31.58	0	6.158	.640	4.0	70
	31.58	4	6.107	.678	3.8	60
May 5, 1939 .....	31.80	0	6.159	.662	4.0	55
	31.80	4	6.139	.681	4.0	55
May 26, 1939 .....	33.76	0	6.140	.704	4.5	55
	33.76	4	6.129	.715	4.3	50

alcohol number was slightly lower in 50 per cent of the trials. The season of the year apparently had no great influence upon the stability of the protein since the slight decrease which occurred was found in alternate months during this study.

The stability of the protein fraction of the condensed skimmilk as determined by the phosphate test was found to have decreased after being held frozen for 4 weeks in 5 of the 9 lots of condensed milk studied. In attempting to correlate the alcohol and phosphate tests, it was found that in 4 of the 9 lots of condensed skimmilk the stability of the protein fraction was found to have decreased by both the alcohol and phosphate tests. In 3 lots the stability as measured by these two tests remained the same, and in the remaining 2 lots of condensed skimmilk the results were inconsistent.

*Comparison of the pH and titratable acidity of the ice cream mixes made from fresh or stored frozen condensed skimmilk.* The pH of the 18 mixes made fell within the range of 6.1 to 6.4 which according to Sommers (11) is the range in pH for normal ice cream mixes. The pH of the ice cream mix containing the condensed skimmilk which had been stored frozen was lower

than that of the ice cream mix made from the same condensed skimmilk while fresh in 5 of the 9 comparisons, while the pH of the condensed skimmilk itself was lowered in 8 of the 9 lots. It was also found that while the titratable acidity of the condensed skimmilk increased after storage, the titratable acidity of the mix was increased in only 2 of the 9 trials by using condensed skimmilk which had been stored frozen 4 weeks at  $-17.8^{\circ}$  C. ( $0^{\circ}$  F.).

*Comparison of the viscosity and resistance to heat coagulation of ice cream mixes made from fresh or stored frozen plain condensed skimmilk.* From the data obtained in determining the viscosity of the ice cream mixes it was evident that the difference in viscosity in the mixes containing fresh condensed skimmilk as compared to the mixes made from the stored frozen condensed skimmilk showed no definite consistent trend due to storing.

The results indicated that the use of the condensed skimmilk which had been frozen had no definite influence upon the resistance to coagulation by heat of the resulting ice cream mixes when the time required to bring about coagulation at  $110^{\circ}$  C. ( $230^{\circ}$  F.) was determined.

*The effect of using fresh or stored frozen condensed skimmilk on the whipping ability of the ice cream mix.* The average whipping ability of the 9 pairs of mixes studied is shown in figure 1. In 6 of the 9 comparative pairs the mix containing the stored frozen condensed skimmilk as a source of added serum solids whipped slower and did not attain as great a maxi-

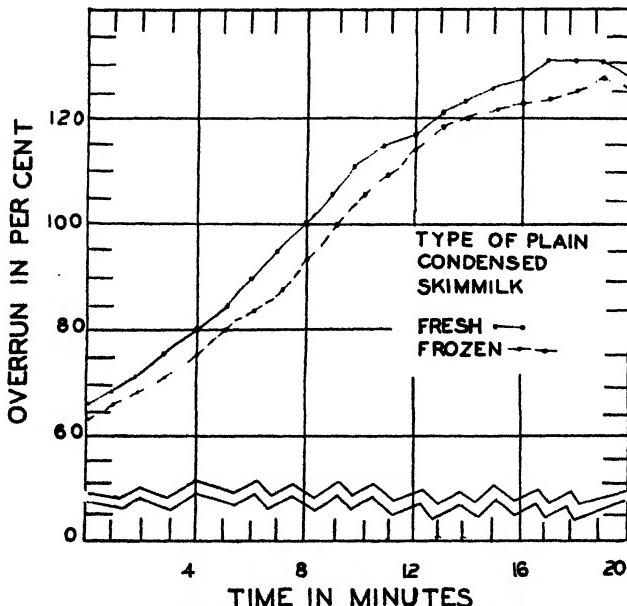


FIG. 1. Whipping ability of ice cream mixes containing fresh condensed skimmilk compared to mixes containing condensed skimmilk stored at  $0^{\circ}$  F. for four weeks.

mum overrun as the mix containing the same condensed skimmilk while fresh. It was interesting to note that at two different times when two mixes were prepared containing the same ingredient other than the condensed skimmilk which was prepared 4 weeks apart, that the whipping properties were the same even though one mix contained fresh condensed skimmilk and the other mix the condensed skimmilk prepared 4 weeks previous and held frozen until used. This would seem to indicate that any difference in the whipping properties of ice cream mixes containing fresh or frozen condensed skimmilk is materially affected by the influence of other ingredients used.

*Effect of using fresh or stored frozen plain condensed skimmilk on the quality of the ice cream.* The ice cream samples were scored after having been in the hardening room for 10 to 14 days. The difference in flavor scores was not significant. Of the criticisms given, four of the samples were criticized for having a slight condensed milk flavor, and in each case frozen condensed skimmilk had been used. The intensity of this flavor was not sufficient to justify the statement that frozen condensed skimmilk should not be used. Seven of the 18 ice creams were criticized for having a slight "cooked" flavor probably due in part to pasteurizing at 160° F. for 20 minutes. In the ice creams made during April and May a feed flavor was very noticeable due to the type of pasture to which the producing animals had access.

In regard to texture, one ice cream was criticized for being "icy." The other 17 were criticized for being either "slightly coarse" or "slightly icy." Our results showed that there was no significant raising or lowering of the score for either body and texture or flavor due to the use of stored frozen plain condensed skimmilk rather than the fresh product.

*Effect of using stored frozen plain condensed skimmilk upon the melting characteristics of the finished ice cream.* Widely varying results were obtained from the melt down tests. The reason for these results might have been the difference in the temperature of the room from month to month which would influence the rate of melting. Two mixes, one containing frozen condensed skimmilk and the other fresh condensed skimmilk, made the same day, frozen the same day, and the melt down tests conducted at the same time gave almost identical results, although significantly different in heat coagulation time and viscosity and similar in other properties studied. This would indicate that the measurements made have no relation to the melting characteristics or the variation in temperature of melting overshadowed differences due to condensed milk ingredients.

#### DISCUSSION OF RESULTS

The chemical analysis of the milk supply used in the manufacture of the condensed skimmilk showed that the milk was normal in regard to per cent of solids, milk fat, lactose, total protein, and ash.

No definite relationship was found between the acidity of the milk products as indicated by the titratable acidity and pH and the protein stability as determined by the alcohol number and the phosphate coagulation time. Rice and Markley (9) found no relationship between natural acidity and coagulability with rennet or alcohol. They did find that these properties ran somewhat hand in hand and they concluded that both depended upon certain relationships between constituents of milk which are independent of acidity. Holm, Webb, and Deysher (2) did not find any correlation between stability of the protein of the fresh milk toward heat and that of the condensed milk manufactured from it. Our results indicated that the heating of whole milk such as that occurring in the process of pasteurization does lower the titratable acidity, due probably to the loss of CO<sub>2</sub>. Whittier and Benton (12) observed a drop in the titratable acidity of milk upon heating and stated that the loss of CO<sub>2</sub> was the factor responsible. The reason for the increase in titratable acidity of the skimmilk upon condensing was no doubt due to the increased solids content.

In this study, no milk was found to produce a condensed skimmilk unstable to freezing and storing for a period of 4 weeks at - 17.8° C. (0° F.). Therefore, it was not possible to state the alcohol number or minimum coagulation time in the phosphate test which would differentiate between the condensed skimmilk suitable for freezing and storing and that skimmilk which would not be suitable because of the instability of the protein fraction.

The freezing and storing of the condensed skimmilk did not alter its physical appearance or body other than to make it slightly sandy. However, as shown in table 2, some changes did occur in the chemical properties. It may be seen that freezing and storing did cause an increase in titratable acidity. The fact that this increase was not consistent may have been due to the original whole milk as well as the condensed skimmilk. It is also rather difficult to explain the drop in pH which occurred in 8 of the 9 lots of condensed skimmilk. A lowered pH would indicate a reduced charge on the protein particle which would reduce the stability somewhat. However, with one exception, the protein stability of the condensed skimmilk as measured by the alcohol test of Dahle and Pyenson (1) was affected only slightly by storing at 0° F. for 4 weeks.

There was no significant trend found in the titratable acidity, pH or viscosity of the ice cream mix due to the changes produced by freezing and storing the condensed skimmilk at 0° F. Winn (13) found that the pH and titratable acidity of the ice cream mixes made from the stored frozen condensed skimmilk were not significantly different from those of ice cream mixes made with the same condensed skimmilk when fresh.

Fluctuations obtained in whipping ability as measured in terms of time to reach 100 per cent overrun do not permit the conclusion that the use of stored condensed skimmilk had any consistent effect on this property. The

score given the ice cream containing the fresh condensed skimmilk agreed very closely to that given the ice cream containing the same condensed skimmilk after it had been stored frozen for 4 weeks at 0° F. This indicated that from the standpoint of body and texture, as well as flavor, condensed skimmilk could be held frozen in storage for 4 weeks at 0° F. and still be used to produce a quality ice cream.

In the measurement of heat stability it was found that 6 of the 9 pairs of mix studied showed less stability when the stored product had been used. Information was not obtained which would explain why that did not hold true in the other 3 pairs of mixes studied. It is also difficult to explain the widely varying results obtained when the ice cream samples were melted down, unless attributed to the fact that the temperature at which the melt down tests were conducted usually varied for each mix of the pair being compared.

It must be recognized that the physical and chemical properties of the skimmilk and cream used in these ice cream mixes may have influenced the properties of the mixes studied.

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## THE INTENSITY AND KIND OF SELECTION ACTUALLY PRACTICED IN DAIRY HERDS\*

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*Iowa Agricultural Experiment Station*

It is important to know how much selection among females actually occurs in dairy herds. Knowing what causes certain cows to remain in herds while others leave, is also important if we are to learn how to make more rapid improvement in dairy cattle through selection.

Attempts to measure the yearly replacements necessary in a herd and thus arrive at the average productive life of dairy cows have been numerous. Spillman *et al.* (19) used this method and estimated the productive life at 4.34 years for cows in Pennsylvania dairy herds and at 4.52 years in Michigan herds. McCandlish (11) gave 3.5 to 4 years as the productive life of dairy cows and Alexander (1) found that the Iowa State College herd had averaged 3.59 years. A report by Smith *et al.* (17) however, showed a shorter productive life (3.17 years) in the milking herds of West Sussex. Lush and Lacy (10) from a study of 500 cows in each of the dairy breeds found the productive life 3.5 years.

A study concerned with the increase in average production of a herd was reported by Gooch (5). She concluded from her study of 1741 eight-month lactation records over a period of 18 years that part of "this (increase) may have been brought about by selecting cows with comparatively high initial yields, since such cows have a high eight-months' total." A study by Plum (13) in Denmark of the Kollekolle herd for the years 1900 to 1934 revealed an 80-kilogram increase in butterfat production of which he attributes 10 kilograms (22 pounds) to the selection of females. This showed an average yearly improvement of .65 pounds of butterfat.

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## MATERIAL AND METHODS

The present study was conducted in order to measure the influence that culling has on the average production of cow testing association herds. "Culls" as defined in this study included all cows which finish a cow testing association year but leave the herd before the completion of the following association year. The "culls" thus included cows leaving herds for all reasons, *i.e.*, those that died, as well as those sold for dairy purposes, for beef, or because of disease, et cetera.

Iowa and Kansas Cow Testing Association data were used in this study. In Iowa, records on 147 herds, representing three herds taken at random from each of 49 Iowa Cow Testing Associations, were used. Records on 37 Kansas herds were also studied. Each herd had been tested continuously from 3 to 6 years in its respective state during this period. The Kansas herds were those having the most complete records for the period studied.

The yearly cow testing association milk and butterfat records were used as a measure of a cow's productivity. After her first calving each cow was considered as "on test" as long as she remained in the herd. There was, of course, variation in whether the cow was actually in milk for the entire 12 months of the period or for only a part of the time, but no corrections for this were made. The age-corrected yearly association records were considered reliable enough for this investigation, inasmuch as they have been shown (7) to repeat themselves in succeeding years about as closely as do age-corrected lactation records.

A total of 4495 different cows were included in the 147 Iowa herds studied. These cows had a total of 8010 records. Within the 37 Kansas herds there were 1883 different cows which had a total of 4087 records.

Inasmuch as the cow's age in most cases was given in even years, it was necessary to use a single age-conversion factor for all animals of a given year of age. Based on findings (12) about freshening ages in Iowa Cow Testing Association herds, the two-year-olds were assumed to be two years and two months of age. Each age group thereafter was likewise considered as the even year plus two months, leaving twelve months as the interval between groups. Bureau of Dairy Industry (9) age conversion factors for these ages were used.

Incomplete records of cows were handled in two ways. If a cow had a previous record and was not in milk at least 10 months during the association year, the incomplete record was not used and her productive ability was based on the previously completed record. Obviously most incomplete records of cows three years and over fell into this group. Two-year-old and a few three-year-old incomplete records were treated differently. For these it was necessary to raise the records to a complete mature-equivalent basis. If this had not been done, any tendency for herd owners to base their culling of heifers on the early production of a first lactation would not have been

found in this investigation, for such heifers have no previous records to indicate their productive abilities.

Conversion factors for raising records to a complete mature basis were used on all records of first-calf cows on test less than 11 months, whether made by animals leaving the herd or by ones remaining. The fact that adjustments were made on those remaining in the herd, as well as on those leaving the herd, would tend to balance any error arising from imperfections of these adjustment factors. These adjustment factors were computed from the Iowa data by choosing at random among cows of a similar age those which had incomplete records their first year but were on test for 12 months the following year.

#### RESULTS OF STUDY

##### *Year to year variations*

Animals leaving herds for all reasons and thus falling into the "cull" classification constituted 28.6 per cent of Iowa herds and 30.9 per cent of Kansas herds. This turnover indicated a productive life of 3.50 years in Iowa Cow Testing Association herds and 3.24 years in Kansas Association herds. These averages would be slightly higher if allowances were made for cows bought after having spent part of their productive lives in other herds, and for cows sold into other herds where they would yet spend a part of their productive lives.

TABLE 1  
*Total cows and culls with percentage of culls by years*

Year	Iowa			Kansas		
	Total Cows	Number Culls	Per cent Culls	Total Cows	Number Culls	Per cent Culls
1930				289	85	29.4
1931	1665	433	26.0	603	195	32.3
1932	2100	537	25.6	778	234	30.1
1933	2009	585	29.1	804	237	29.5
1934	1600	518	32.4	838	309	36.9
1935	636	215	33.8	775	204	26.3
Total or average	8010	2288	28.6	4087	1264	30.9

$$X^2 = 34.8, P < .001$$

$$X^2 = 24.4, P < .001$$

Highly significant differences<sup>2</sup> were found between the amount of culling by years (table 1). The lowest percentage of culls during the years studied was for Iowa in 1932 when 25.6 per cent of the cows were culls, while the highest percentage of culling occurred in Kansas herds during the drouth year of 1934 when 36.9 per cent of the cows became culls. Likewise, the drouth caused heavy culling in Iowa herds with 32.4 per cent culled in 1934 and a still higher percentage (33.8 per cent) leaving herds in 1935. Differ-

<sup>2</sup> Throughout this study, probabilities of .01 or less were considered as highly significant while significant probabilities were taken as those lying between .05 and .01.

ences in assigning individual cow testing association records to a given year are thought to account for these two years of heavy culling in Iowa herds as contrasted to the one year (1934) in Kansas.

TABLE 2  
*Averages and differences between production of culls and non-culls*

	All cows		Non-culls		Culls		Difference: non-culls minus culls		Difference: all cows minus non-culls*	
	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat
Iowa .....	8985	359	9355	374	8061	320	1294	54	370	15
Kansas ....	8379	348	8633	362	7812	316	821	46	254	14

\* Selection differential.

Culls on the average produced less milk and butterfat for each of the years studied than did the non-culls. In Iowa herds the average non-cull excelled the average cull (table 2) by 1294 pounds of milk and 54 pounds of butterfat annually. Obviously there was no clear-cut division between the productive levels of the two groups (see figure 1), for among the culls were some good producers and among the non-culls were some poor producers. Kansas non-culls produced an average of 821 pounds of milk and 46 pounds of butterfat more than the culls. Variation as between years ranged from a difference in milk production of 752 pounds (Kansas, 1933) to 1837 pounds (Iowa, 1931). Butterfat differences varied from a low of 37 pounds (Kansas, 1934) to a high of 73 pounds (Iowa, 1931). In both states the heavy culling attributable to the 1934 drouth reduced the production spread between the two groups.

The spread between the butterfat production of culls and non-culls when tested for significance by the analysis of variance (18) showed highly significant differences for each of the years studied within each state. The portion of the total variance attributed to differences between the two groups varied from 3.3 to 10.1 per cent for Iowa and from 2.7 to 5.8 per cent for Kansas.

The selection differential which is computed by determining the difference between the production of non-culls and all cows (table 2), shows how much higher the herd average would have been in that year if the culls had been removed before the year began. For the five years in Iowa this selection differential averaged 370 pounds of milk and 15 pounds of butterfat, and in Kansas the six year average was 254 pounds of milk and 14 pounds of butterfat.

#### *Herd differences in culling*

Differences in herd practices and feed available, as well as the owner's financial needs, are among the factors which, in addition to differences in the

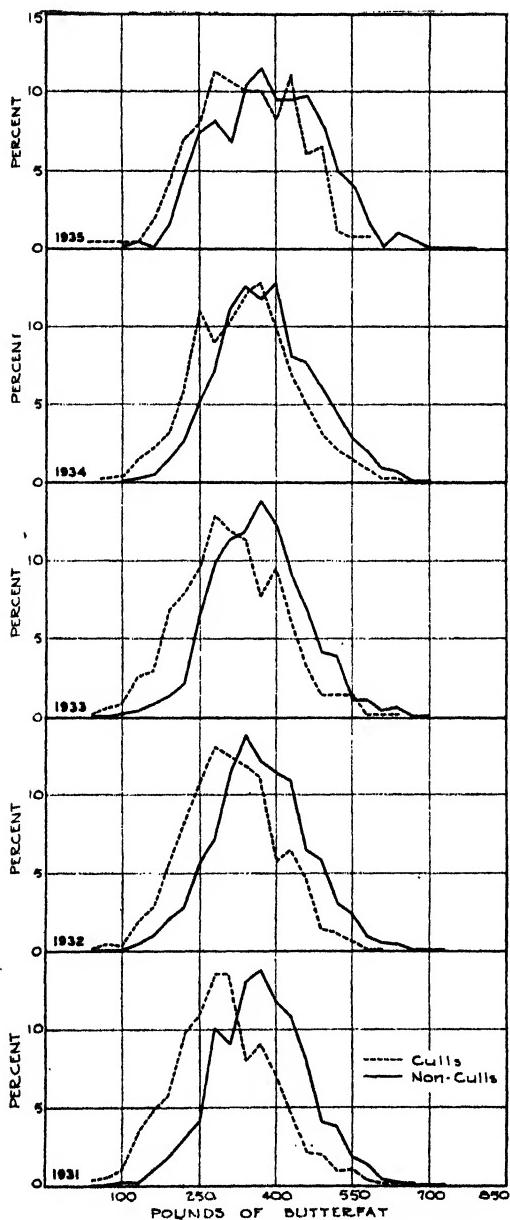


FIG. 1. Iowa non-culls and culls grouped according to butterfat production level.

percentages of low-producing and diseased cows, could cause variations in the amount and intensity of culling between herds. Actual differences found in percentage culled between the 37 Kansas herds varied from a low of 17 per cent to a high of 49 per cent. The herd differences in percentage culled were highly significant and gave evidence that factors affecting the herd as a whole caused some herds to have a high percentage of culls and other herds to have a low percentage of culls.

In general, there was evidence that Kansas herds having a high percentage of culls also had a wide spread between the production of non-culls and culls. A few striking exceptions to this trend were found, however, and no effort was made to measure its statistical relationship.

The selection differentials by herds also showed a wide range in Kansas. In one case the entire herd actually averaged 66 pounds more milk than the non-culls. The most evidence of culling for milk production was for a herd whose non-culls produced 740 pounds more milk on an average than all the cows. The range in butterfat selection differential was from zero to 33 pounds.

The method of fitting constants (21) was used in the Iowa study to remove the effects of disproportionate frequency and thus to permit testing the data for "interaction" between herds and differences in the butterfat production of non-culls and culls. A real interaction was found for each of the five years studied. This means that among the 147 herds some were culled for production much more severely than others.

Inasmuch as positive evidence was found in the Iowa and Kansas studies that the amount as well as the intensity of culling for production varied from herd to herd, this phase of the investigation was pushed further with Iowa data in an effort to learn whether these characteristics peculiar to a herd continued year after year.

The first phase of this investigation concerned the selection differential, *i.e.*, the amount that non-culls excelled all cows. Low positive correlations existed between the selection differentials of a herd in consecutive years, ranging from .02 to .13 with an average of .09.<sup>3</sup> They were not statistically significant. For non-consecutive years the correlations varied from .11 to .34 and averaged .23, which was highly significant. Why non-consecutive years showed a higher degree of association than consecutive years is not readily apparent. One plausible explanation is that a man might severely cull his herd for production one year but, because of a lack of replacements the next year, be obliged to wait until the third or fourth year to duplicate the process.

As might be expected, close relationships were found between the percentage culled and the selection differential. The correlation ranged from .11 to .56 and averaged .36.

<sup>3</sup> All correlation averages were computed by the Z method, Fisher, (4).

The herd's average butterfat production did not, however, reveal any such consistent relationship to the selection differential and had two negative correlations (-.03 and -.02) and two positive correlations (.18 and .09), averaging .06 for the four years studied. These results would indicate that the herds with high herd averages were able to practice on an average as intense, if not slightly more intense selection for high production as did herds with lower butterfat averages.

Low, but non-significant, relationships were found between the size of herd and the selection differential, indicating that as the size of the herd increased the selection differential tended to be slightly larger. The correlations by years varied from .02 to .47 and averaged .13. *A priori* it would not seem that size of herd would have this effect, since the percentage of replacements would be about the same in large and in small herds. There might, however, be management practices associated with herd size which would help or hinder culling in herds of some sizes more than in herds of other sizes.

Correlations between consecutive years for percentage of herd culled gave further evidence as to the degree to which voluntary selection was a characteristic of herd management. Low correlations averaging .10 and ranging from zero to .15 were found. Percentage of animals culled was obviously not entirely the result of voluntary culling by men in charge of herds but includes also a great deal of irregular and involuntary culling governed partially by the number of replacement animals available.

#### *Breed differences in culling*

A comparison of the percentage of purebreds, grades and scrubs culled in Iowa and Kansas Association herds showed (table 3) one striking difference as between states. In Iowa the percentage culled among purebreds (28.2 per cent) was not significantly different from the percentage culled (28.7 per cent) among the grades. Kansas, on the other hand, with 26.7 per cent culled among purebreds and 35.4 per cent culled among grades, displayed almost one-third more culling among grades than among purebred animals. The high percentage of culling of Kansas grades could partially have been caused by the presence of a number of herds that had both grades and purebreds. The owners of these herds may have been eager to convert their herds rapidly to wholly purebred ones and, therefore, may have done most of their voluntary culling of grades. The 80 scrubs in Iowa herds were culled more heavily (table 3) than either the purebreds or grades.

The intensity of culling as measured by the production spread between the non-culls and culls was greater in each state for purebreds than for grades (table 3). In Iowa the purebred non-culls excelled the purebred culls by 2696 pounds of milk and 57 pounds of butterfat, whereas the grade non-culls produced 1321 pounds of milk and 53 pounds of butterfat more

TABLE 3  
*Intensity and homogeneity of culling among purebreds, grades and scrubs*

	Iowa				Kansas			
	Cows		Production difference of non-culls and culls		Cows		Production difference of non-culls and culls	
	Number studied	Per cent culled	Milk	Fat	Number studied	Per cent culled	Milk	Fat
Purebred .....	2918	28.2	2696	57	2124	26.7	776	49
Grades .....	5011	28.7	1321	53	1953	35.4	708	41
Scrubs .....	80	31.2	1814	62	*			

\* Three scrubs, 3 Brown Swiss and 4 Red Polled cows were eliminated from the Kansas study of breeds because there were so few in each class.

than the grade culled. Kansas data revealed this same trend with the purebreds excelling in both comparisons between non-culls and culled with a milk yield difference of 776 pounds for purebreds and 708 pounds for grades, and a butterfat yield difference of 49 pounds for purebreds and of 41 pounds for grades.

Between-breed differences in amount of culling were found in both the Iowa and Kansas studies, yet the results were not consistent as between states. Iowa herds culled a strikingly large percentage of grade Guernseys (32.6 per cent) and a relatively small percentage of purebred Guernseys (18.1 per cent), while in Kansas purebred Holsteins and Grade Ayrshires with 29.5 per cent and 49.0 per cent culled ranked first, respectively, in culling among the purebred and grade groups. The least amount of culling among Kansas breeds was for purebred Jerseys with 22.4 per cent culled. Chi-square values derived from tests for homogeneity of culling between breeds gave significant figures for each state. These values would likely have been less had the influence caused by the general differences between herds been first removed, for it has already been shown that factors affecting the herd as a unit had an influence on the amount of culling done. Inasmuch as most herds consisted largely of one breed, the heavy culling of a herd would exert its culling influence largely on one breed.

Differences between the production of non-culls and culled by breeds were irregular as between states, thus failing to indicate that one breed selected more closely for either high milk or high butterfat production than did the others.

#### *How months in milk affected culling*

The production records used in this study were for a cow testing association year rather than for a lactation. The period that the cows were in milk showed (to the nearest month) the time during an association year that the

cow was in production. The months stated thus represented the part or parts of one or of two lactations that a cow was milked.

When the cows were grouped by months in milk, differences in the percentage culled during the next year were significant in both states (top half of table 4). Before making these comparisons the two- and three-year-olds with incomplete records were removed from both the cull and non-cull groups, so that no discrepancy would be introduced by their inclusion. A distinct tendency was found to cull heavily the cows that were in milk only a small part of the preceding year and to retain a higher than average percentage of those in milk ten or more months. Forty-eight per cent of the Iowa cows and 40.9 per cent of the Kansas cows in milk five or less months were culled. This was in contrast to an average culling among these groups of 26.5 per cent in Iowa and 29.0 per cent in Kansas. Cows in milk eleven months experienced the least culling, 24.3 per cent in Iowa and 26.8 per cent in Kansas.

The milk and butterfat production differences between non-culls and culls (top half of table 4) showed no uniform trend either within states or between states that could be ascribable to months in milk. For certain years, however, there was evidence that a definite interaction did exist between months in milk and the butterfat production spread between culls and non-culls. While not tested in the Kansas study, evidence of such interaction was found in Iowa for 1931, 1932, and 1933. The years 1934 and 1935, however, showed a definite lack of interaction. The lack of relationship in 1934 and 1935 may have been due to the influence of the drouth which could have upset normal culling practices.

The culling among two- and three-year-olds (bottom half of table 4) having incomplete first lactations averaged 35.0 per cent in Iowa and 37.1 per cent in Kansas, and was significantly heavier than the 26.5 per cent and 29.0 per cent respectively shown in these states for other cows (top half of table 4). The culling of these young cows was comparatively heavy for each of the months-in-milk groups with no apparent uniform trend as between states.

The production spread between culls and non-culls, as with the percentage of culling, was also greater for these two- and three-year-olds with incomplete records (raised to a full-time basis) than for other cows—in Iowa 2017 pounds of milk and 74 pounds of butterfat as compared to 559 pounds of milk and 40 pounds of butterfat for other cows, and in Kansas 970 pounds of milk and 56 pounds of butterfat as compared to 717 pounds of milk and 41 pounds of butterfat for other cows. This evidence of early selection based on the first production records of cows is further supported by the larger production spread between the culls and non-culls during the early part of these first lactations (bottom half of table 4). Within each state this spread was the greatest for one month in milk, with those in milk two

TABLE 4  
The amount and intensity of calling as influenced by months in milk during preceding year

Mos. in milk	Iowa			Cows			Kansas		
	Cows		Production difference of non-culls and culls	Cows		Production difference of non-culls and culls			
	Number studied	Per cent culled	Milk	Fat	Number studied	Per cent culled	Milk	Fat	
Complete records									
2	3	4	48.0	4450	245	3	40.9	1400	12
3	4	5	25	1567	60	22	-	-1350	-60
4	6	7	12	-1860	-46	4	-	-1500	-60
5	5	6	-	-2613	10	12	-	-1213	-21
6	6	7	26	830	33	24	-	-730	-28
7	7	8	66	657	35	38	41.7	596	19
8	8	9	304	857	38	167	35.9	1155	52
9	9	10	733	28.8	780	30	406	31.3	586
10	10	11	2361	25.8	1048	45	1136	28.3	29
11	11	12	1669	24.3	567	33	889	26.8	512
12	12	Total or average...	839	25.4	706	37	437	27.7	922
Total or average...	6023	26.5	559	40	3119	40	29.0	5	51
Total or average...	1983	35.0	2017	74	968	74	717	41	82
$X^2 = 35.9, P < .001$					$X^2 = 17.3, P < .01$				
Incomplete 2- and 3-year-olds—records raised to full-time basis									
1	1	2	137	37.4	119	108	35.2	1754	108
2	2	3	239	33.5	2360	84	126	956	58
3	3	4	231	35.9	1719	77	118	1288	66
4	4	5	244	34.8	2403	84	642	30.5	1499
5	5	6	199	38.2	2642	96	36.0	35.2	36
6	6	7	243	39.5	2008	73	45.7	866	47
7	7	8	181	34.3	1936	78	88	36.4	695
8	8	9	223	38.6	1639	50	68	33.8	47
9	9	10	163	31.3	600	28	72	403	40
10	10	Total or average...	123	24.4	741	18	90	413	48
Total or average...	1983	35.0	2017	74	968	74	717	1647	82
Total or average...	1983	35.0	2017	74	968	74	717	970	56

and three months also showing a wide production spread between the two groups.

These findings warrant the conclusion that much early culling based on production takes place during the first lactation of two- and three-year-olds in both Iowa and Kansas Cow Testing Association herds.

#### *Age and its influence on culling*

The influence of age on the number culled and on the production spread between culls and non-culls was studied by using the ages of the cows given (in whole years) for the year prior to culling (the incomplete record two- and three-year-olds excepted). This placed a large portion of the cows in an age group one year less than the actual age when they left the herd.

Both Iowa and Kansas herds showed a definite effect of age of cows on culling rates. (See figure 2 for distribution by ages of Iowa culls and non-culls.) In Iowa the culling rates among cows less than eight years old were

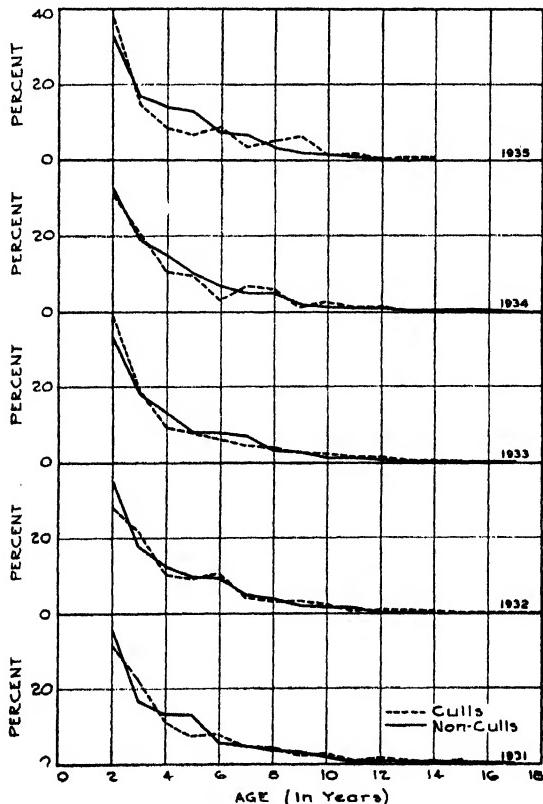


FIG. 2. Distribution of culls and non-culls by ages (Iowa).

the heaviest for three-year-olds with 31.3 per cent (table 5) of their number culled. The two-year-olds were second with 28.1 per cent culled. Kansas herds culled the two-year-olds most heavily (31.6 per cent) and the three-year-olds second (30.4 per cent). An important part of this heavy culling of two- and three-year-olds as previously shown was among those with incomplete first-lactation records.

TABLE 5  
*The percentage of culled by ages (Ages given for year prior to culling)*

Age (years)	Iowa				Kansas			
	Cows		Production dif- ference of non- culls and culled		Cows		Production dif- ference of non- culls and culled	
	Number studied	Per cent culled	Milk	Fat	Number studied	Per cent culled	Milk	Fat
2 .....	2705	28.1	1659	63	1325	31.6	1107	57
3 .....	1500	31.3	1764	75	723	30.4	1102	54
4 .....	995	23.2	649	34	489	27.2	766	53
5 .....	800	25.0	647	38	403	26.8	549	37
6 .....	616	27.4	1200	47	337	26.1	598	27
7 .....	448	26.3	993	46	274	29.6	192	27
8 .....	337	31.8	838	32	216	32.9	321	41
9 .....	231	34.2	744	40	135	42.2	- 166	35
10 .....	164	38.4	1367	52	72	44.4	1334	36
11 .....	94	33.0	1422	60	46	45.7	569	20
12 .....	60				26			
13 .....	31				18			
14 .....	15				11			
15 .....	8	50.7	197	12	7	50.7	1682	54
16 .....	2				4			
17 .....	2				1			
18 .....	2							
Total or average	8010	28.6	1294	54	4087	30.9	821	46
X <sup>a</sup>		69.2				47.4		
d/f		10				10		
P		< .001				< .001		

Advancing age caused a decided increase in culling as evidenced by the heavy culling of cows eight or more years of age (table 5). Especially heavy culling took place among cows twelve years or older, with an average of 50.7 per cent of those leaving the association herds in both Iowa and Kansas.

The production differences between the non-culls and culled by age groups (table 5) were large for both milk and butterfat production for the heavily culled two- and three-year-old groups. In Iowa the three-year-olds were the most intensely culled, their non-culls excelling their culled by 1764 pounds of milk and 75 pounds of butterfat. In Kansas the two-year-old non-culls had the largest spread over culled of any of the age groups with 1107 pounds of milk and 57 pounds of butterfat. In Iowa the two-year-olds ranked sec-

ond, while in Kansas the two classes were reversed with the three-year-olds second. Irregular production differences having no particular trend were found for groups 12 years old or over.

### *The influence of body weight*

Weight was studied as a possible factor which might have influenced selection, inasmuch as various studies have shown that larger cows generally produce more milk and butterfat than do smaller ones.

The study of the influence of weight on culling was confined to Iowa data where the estimated weights on the yearly reports of the testing supervisors were used. Such data were available on approximately 80 per cent of the cows within the 147 herds. Although these weights were only estimates of the herd owner and testing supervisor, and of course would vary from year to year, they did furnish some information on the general size of the animals.

Indications were found (table 6) that cows weighing 600 pounds or less were culled heavily, for 40 per cent of the cows falling into this group were culled as compared to an average of 28.8 per cent for the 7436 cows. Also, the cows weighing 1500 pounds or more showed a high percentage culled, with 35.3 per cent of them leaving the herds.

TABLE 6

*Percentage culled and production differences between culls and non-culls as influenced by body weight (Iowa herds only)*

Body weight (pounds)	Cows		Production difference of non-culls and culls	
	Number studied	Per cent culled	Milk	Fat
600 or less .....	130	40.0	1041	58
700 .....	289	30.8	746	42
800 .....	1046	28.0	991	47
900 .....	1031	27.4	985	47
1000 .....	2048	28.0	1421	58
1100 .....	1103	27.9	1675	65
1200 .....	1026	31.0	1389	55
1300 .....	365	29.6	1754	69
1400 .....	282	25.9	1273	57
1500 and over .....	116	35.3	69	9
Total or Average .....	7436	28.8	1282	54

$$X^2 = 15.4, P .08$$

The significance of the differences found in number culled by weight groups when studied by chi-square for individual years left much doubt as a definite relationship of culling to body weight. In only one year (1933) was the probability less than .05 that culling intensity was independent of weight. When all data were combined for the five years the probability was .08. Only the extreme classes (600 pounds or less and 1500 pounds and

over) seem likely to have been culled with an intensity really different from that in other weight classes.

Differences between the production of non-culls and culls by weight groups (table 6) displayed little evidence of a trend. Only one year, 1931, had a significant interaction between weight and the difference in production. This was offset by the years 1932, 1933, and 1934 where the mean square due to interaction was actually less than that within the groups. Even though herd owners did cull more heavily the extremely light and extremely heavy cows, the production differences between the non-cull and cull groups within the various weight classes did not vary in a regular way with weight of the cow.

#### *Why cows were culled*

For the Kansas study herd owners furnished through their testing supervisor information as to why cows left the herd. In many cases the reason given had been recorded in the herd record book, thus representing the reason given at the time the animal became a cull. In instances where this notation had not been made, the owner relied on his memory for the reason for culling. In case the reason could not be recalled, it was classified in the questionnaire as "reason unknown." Fifty animals representing 4 per cent of the culls were thus classified.

Cows sold because of low production accounted for 9.4 per cent of total cows or 30.5 per cent of the 1264 culls (table 7), i.e., 30.5 per cent equals 7.1

TABLE 7  
*Why Kansas cows were culled and production level of each group*

Reason	Number culls	Per cent of		Av. milk	Av. fat
		Total culls	Total cows		
Bang's disease .....	168	13.3	4.1	7873	325
Udder trouble .....	133	10.5	3.3	8692	347
Sterility .....	92	7.3	2.2	7321	321
Died .....	83	6.6	2.0	8635	349
Misc. diseases .....	10	0.8	0.3	9100	413
Tuberculosis .....	8	0.6	0.2	7938	358
Total diseased .....	494	39.1	12.1		
Low producers sold for beef .....	296	23.4	7.2	7196	289
Low producers sold for dairy .....	90	7.1	2.2	6100	247
Good producers sold for dairy .....	240	19.0	5.9	8472	327
Old age .....	61	4.8	1.5	6328	341
Accidents .....	30	2.4	0.7	8360	334
Misc. reasons .....	3	0.2	0.1	7567	300
Reason unknown .....	50	4.0	1.2	7146	312
Grand total .....	1264	100.0	30.9	7812	316

per cent sold for dairy plus 23.4 per cent sold for beef. The low producers sold for dairy purposes had the lowest average production of any group—

6100 pounds of milk and 247 pounds of butterfat! The low producers sold for beef ranked second low in butterfat yield with 289 pounds of butterfat.

A total of 494, representing 39.1 per cent of the culls (12.1 per cent of total cows), left because of disease. Among the diseased groups those leaving because of sterility and Bang's disease produced the lowest yields of butterfat, 321 pounds and 325 pounds, respectively. Those afflicted with udder troubles averaged 347 pounds, just one pound less than the average of all cows.

Cows classed as good producers and sold for dairy purposes represented 19 per cent of the total culls. Their average production was 8472 pounds of milk and 327 pounds of butterfat. This was 93 pounds in excess of the average milk yield but 21 pounds less than the average butterfat yield of all cows. These dairymen apparently exercised some selection for higher butterfat percentage when animals were sold for dairy purposes.

The findings relative to percentage culled for various reasons (table 7) are about the same as the results reported (15) for a three-year period (1935-1937) on all Iowa and Kansas cow testing association herds, and are in close agreement with the Bureau of Dairy Industry's report (2) on a study made of 114,135 cows in 18 states. Culling for low production was strikingly similar between the three studies, ranging from 30.5 per cent (this study) to 35 per cent of the total culls. Losses due to disease in this study (12.1 per cent of total cows) coincided closely with the 13.5 per cent reported by Smith (17) in West Sussex cattle.

#### *Age and the culling of low producers*

As has been shown (table 7) 386 of the 1264 culls, or 30.5 per cent of all culls, were sold because of low production. When these 386 animals were classified by ages (table 8) a preponderance of them fell into the younger groups. Two-year-olds made up 40.2 per cent of their total, three-year-olds 23.8 per cent and four-year-olds 9.1 per cent, while only 4.4 per cent of them were over eight years of age.

TABLE 8  
*Ages and production level of cows sold because of low production*

Age (years)	Number cows	Per cent of all culls	Average production	
			Milk	Fat
2	155	40.2	7055	278
3	92	23.8	6499	257
4	35	9.1	6540	260
5	26	6.7	7719	307
6	28	7.2	7278	311
7	14	3.6	7171	287
8	19	4.9	6647	265
9 and over	17	4.4	7541	292
Total or average	386	100.0	6942	277

The average mature-equivalent butterfat productive level of the various age groups (table 8) shows three-year-olds lowest with 257 pounds, while the two-year-olds averaged 278 pounds, and the four-year-olds 269 pounds. The 19 eight-year-olds were second low with 265 pounds while the highest average was registered by the 28 six-year-olds with 311 pounds. This group still produced five pounds less than the 316 pound average of all culled.

The indication that most of the culling for low production takes place early in a cow's productive life was confirmed in this portion of the study. Age relationships to culling, when all culled were considered, indicated this same trend (table 5).

#### *When cows are culled*

A sample of 414 culled having records (or possible records) through the two-, three-, and four-year-old periods were chosen in an effort to learn about the length of productive life and the number of freshenings of cows which are culled at early ages. Obviously many of these young cows did not have records for each of the three years studied, inasmuch as they became culled before they completed the three years in herds. In every case, however, care was taken to choose only records of cows that were in herds that were tested for three years following the beginning of these two-year-old records, so as to permit the securing of all their records, irrespective of whether they stayed in the herd for part or all of the three years.

The distribution of these culled for number of freshenings to time of culling showed that 50.7 per cent of the 414 had freshened but once, 32.8 per cent had freshened twice, 12.8 per cent freshened three times, and 3.6 per cent had four freshenings before being culled. The average was 1.7 freshenings to time of culling.

Of these 414 culled, 156 were cows that were classified by the owner as culled because of low production and sold either for beef or dairy purposes. For these low producers, the percentage distributions by number of freshenings were very nearly the same as those of the entire sample. Likewise, the average of 1.6 freshenings per cow is not very different from the average of 1.7 found for the entire 414 culled.

The average number of months elapsing from first freshening to time of culling for the 414 culled was 17.3 months. For the 156 culled that were sold because of low production it was 14.7 months, showing a greater difference than was apparent in the comparison between the two groups on number of freshenings.

The average period from last freshening to time of culling was 5.3 months for the entire group and 5.2 months for the low producers. This showed that the time of culling averages around the center of a cow's last lactation. The distribution of cows by months showed little tendency for the culled to have been sold mostly during specific months.

*The effect of selection on inheritance of herds*

If the selection differential represented only permanent differences between the cows, if these differences were 100 per cent hereditary, and if the choice of bulls represented culling of the same intensity, then it could be said that culling caused an annual improvement in the heredity of Iowa herds of 370 pounds of milk and 15 pounds of butterfat, and in Kansas of 254 pounds of milk and 14 pounds of butterfat (table 2). Unfortunately, the answer is not this simple. In the first place, the selection studied was only of cows and they contribute only one-half the genes to the next generation. Secondly, one record on a cow is only a partial indication of her real producing ability, and thirdly, only a fraction of the measured differences in cows is hereditary.

Now, if heredity were the only thing which caused records of the same cow to be alike, the intra-herd correlation between consecutive records of cows would represent the hereditary portion of the differences between single records of cows. In Iowa this correlation for butterfat yield was .38 for non-culls and .31 for culls and in Kansas .32 for non-culls and .27 for culls. These results are slightly lower than the .40 found by Plum (12) between consecutive lactation records in Iowa Cow Testing Associations. As was pointed out in that study, it is improbable that this 40 per cent (.40) of the intra-herd differences was entirely caused by differences between the heredity of cows, for there could exist intra-herd correlations between the environment to which the same cow had been exposed for different years. There might exist also a correlation between the inheritance of a cow and her environment, as when the cow with the best inheritance is given the best treatment.

The fact that culls had intra-herd correlations significantly lower than those for non-culls gives evidence that the butterfat differences actually found between the records of non-culls and culls for the year before the culls left the herd were somewhat larger than the real inherited differences. This fact plus the knowledge that all cows eventually become culls makes it logical to consider the intra-herd correlations between consecutive butterfat records of culls as the upper limit in heritability of butterfat yield, *i.e.*, .31 in Iowa and .27 in Kansas.

As a more dependable estimate of the importance of heredity in causing the observed intra-herd differences, dam-daughter correlations were computed on an intra-sire basis. The first record of each animal in dam-daughter pairs was used, so as to be affected as little as possible by selection. Resulting correlations before removing the effect of sire (including herd) were, in Iowa, .51 for milk and .28 for butterfat and, in Kansas, .57 for milk and .37 for butterfat. These results for milk are comparable to those of Gowen (6) who reported Jerseys at .30 and Holsteins at .50. On an intra-sire basis the correlations in the present study reduced for Iowa, to .10 for

milk and .07 for butterfat and, in Kansas, to .12 for milk and .02 for butterfat.

Doubling intra-sire dam-daughter correlations and adjusting for differences in the variance of daughters and dams provides an estimate of the importance of heredity in causing differences between records of cows kept in the same herd and mated to the same sire. This is identical with computing intra-sire regression of daughters' records on dams' records and doubling that in accordance with the principle of "diallel crossing" (14). Questions of whether the mating systems are random, inbreeding, or assortive mating based on somatic resemblance, are avoided by this method. This analysis yields only that fraction of the variance which can be expressed as due to additive gene interactions, as dominance deviations do not contribute to dam-daughter correlations and only a small part of the epistatic deviations do. In this narrow sense the fraction of the variance which is hereditary becomes:

$$\text{Iowa: } 2 \times .10 \sqrt{\frac{428.0}{376.8}} = .21 \text{ (milk)} \quad 2 \times .07 \sqrt{\frac{65.8}{70.9}} = .13 \text{ (fat)}$$

$$\text{Kansas: } 2 \times .12 \sqrt{\frac{654}{580}} = .25 \text{ (milk)} \quad 2 \times .02 \sqrt{\frac{108}{66}} = .05 \text{ (fat)}$$

Now if one adds to each of the above portions of the variance one-fourth of their respective values in line with Wright's (20) idea as the portion due to dominance or increases their value 50 per cent in line with Fisher's idea (3) in allowing for dominance, the importance of heredity (in this broader sense) increases. Wright's method would establish the lower limits in heritability which in this case would be, for milk, .26 (Iowa) and .30 (Kansas) and, for butterfat, .16 (Iowa) and .06 (Kansas).

Considering the intra-herd correlations between consecutive records of culls to be the upper limits of heritability for butterfat and the figures from the Fisher method as upper limits for milk (where the correlation between consecutive records was not studied), the range becomes:

$$\begin{array}{ll} \text{Iowa: } .26 \text{ to } .31 \text{ (milk)} & .16 \text{ to } .31 \text{ (fat)} \\ \text{Kansas: } .30 \text{ to } .38 \text{ (milk)} & .06 \text{ to } .27 \text{ (fat)} \end{array}$$

Before extracting from the selection differentials the above portions attributed to heredity, it is necessary to correct the differential itself to allow for the low production of certain culls, e.g., old and diseased cows during the last year they were in the herd. As evidence of this, among 1129 cows in Iowa herds all through 1933 and 1934, 368 became culls in 1935. During 1934 the non-culls averaged 14 pounds more butterfat than all cows but in 1933, a year earlier, this difference between the same cows was only 9 pounds.

To allow for this non-hereditary portion of the selection differential only two-thirds of the actual amount found was used in computing the hereditary portion, thus becoming, in Iowa, 247 pounds of milk and 10 pounds of but-

terfat and, in Kansas, 167 pounds of milk and 9.3 pounds of butterfat. Now if these characters were 100 per cent hereditary, only one-half of this would pass on to the next generation, inasmuch as each parent contributes equally to the offspring. Extracting this one-half from each (corrected) selection differential and applying the factors (cited above) representing the range in estimates of heritability, results in a net yearly hereditary improvement in milk yield of from 32 to 38 pounds in Iowa and from 25 to 32 pounds in Kansas. In butterfat yield the yearly improvement would be .80 to 1.55 pounds in Iowa and .28 to 1.25 in Kansas.

The indicated annual hereditary improvement in cow testing association herds thus resulting from the culling of cows as shown here would range (considering the results from both states) between 25 and 38 pounds of milk and .28 and 1.55 pounds of butterfat.

These culling benefits are not very different from the results reported by Plum (13) in his study of records covering 35 years in the Danish Kollekolle herd. He found an improvement of 80 kilograms of butterfat of which he stated that not over 10 kilograms was hereditary improvement brought about by the selection of females. This amount (22 pounds) would represent an average of .64 pounds per year, which falls between the limits of improvement found for butterfat in this study.

#### SUMMARY

In a study of 147 Iowa and 37 Kansas Cow Testing Association herds it was found that the annual exodus of cows averaged 28.6 per cent for five years in Iowa and 30.9 per cent for six years in Kansas. The 1934 drought appreciably increased culling in both states.

For each year in both states the non-culls averaged higher in production than did the culls. The average production spread between the two groups was 1294 pounds of milk and 54 pounds of butterfat in Iowa and 821 pounds of milk and 46 pounds of butterfat in Kansas.

Herd differences were found both in percentage culled and in intensity of selection for high production.

Heavier than average culling was found among cows that only milked a few months the previous year. In Iowa herds 48 per cent of those cows that milked five or less months were culled, and in Kansas 40.9 per cent of this group were eliminated.

Heavy culling during the first lactation of two- and three-year-olds was found in each state with 35.0 per cent of such cows (having incomplete records) leaving Iowa herds and 37.1 per cent leaving Kansas herds. These culls showed appreciably lower production records than did other culls.

Ages of cows had an important relationship to culling. Two- and three-year-olds were heavily culled and showed an approximate 25 per cent increase in spread between the production of non-culls and culls as compared

with the average of all age groups. An average of 50.7 per cent of cows 12 years of age and over was culled in each state.

No consistent relationship was found between breeds and culling.

Iowa cows having estimated body weights of 600 pounds or less and 1200 pounds or more were heavily culled, yet too few cows fell into these two classes to permit definite conclusions to be drawn.

Reasons given for culling Kansas herds showed that 39.1 per cent of culls left because of disease. Only 6.6 per cent of the culls died. A total of 30.5 per cent of culls was sold because of low production and good producers sold for dairy purposes accounted for 19.0 per cent. Those sold because of sterility and Bang's disease showed the lowest previous years' production among the diseased cows. Among all groups the low producers sold for dairy purposes produced the least butterfat with low producers sold for beef next above them.

A study of 414 culls followed through the heavy culling ages, i.e., two, three, and four years, revealed that the average number of freshenings to culling was 1.7; the months from first freshening to culling, 17.3; and months from last freshening to culling, 5.3.

The estimated yearly hereditary increase in herd average expected to result from the selection found in this study ranged from 25 to 38 pounds of milk and from .28 to 1.55 pounds of butterfat.

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# RUMEN DIGESTION IN THE BOVINE WITH SOME OBSERVATIONS ON THE DIGESTIBILITY OF ALFALFA HAY<sup>1</sup>

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The importance of rumen digestion has been recognized for a long time but it has been only within recent years that dependable experimental evidence pertaining to rumen physiology has been obtained. Even now, little is known of the significance of rumen digestion when compared with the total digestion that occurs in the passage of feeds through the digestive tract. Recently, however, *in vivo* chemical studies of the digestion of various nutrients within the rumen have been undertaken. Silver (1) studied the digestion and absorption of alfalfa hay by removing the rumen contents at the time of feeding and at 2-hour intervals thereafter. He interpreted his results by comparing the various values for percentage composition of the rumen contents as the period of digestion progressed. Kick and Gerlaugh (2) studied the effect of the preparation of alfalfa hay on rumen digestion and reported the percentage of the total daily intake that was represented by the rumen ingesta when removed 24 hours after feeding. In both of these investigations it was found that protein disappeared rapidly from the rumen while fiber disappeared slowly. Quittek (3) and Krzywanek and Quittek (4) compared the percentage composition of the rumen contents removed at 3-hour intervals with that of the hay. They observed that the percentages of nitrogen, crude fiber and crude fat in the dry matter of the rumen increased as digestion progressed. The increase in these nutrients was attributed to the rapid disappearance of carbohydrates by rumen fermentation. Although all of these methods have served a purpose, they are inadequate in making studies of rumen digestion because the results can only be interpreted on a comparative basis and no idea of the quantitative digestion can be obtained.

*In vitro* studies of the products of cellulose fermentation by rumen and intestinal flora have been numerous. The recent studies of Woodman and Evans (5) and Pochon (6) demonstrated the formation of the lower fatty acids from the hydrolytic decomposition of cellulose and suggested that these acids may be the products of rumen digestion. Acetic and butyric acids were produced in considerable amounts while lactic, pyruvic, propionic, formic, and valeric acids and glucose were produced in smaller amounts. The findings of Trautmann (7) indicate that the rumen, reticulum and

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omasum are capable of rapid absorption of water and substances in solution. Davey (8) also obtained evidence of absorption from the stomach compartments of ruminants.

It has been generally assumed that the various nutrients of feed-stuffs are less digestible at the higher planes of nutrition. Watson and co-workers (9) have pointed out that the data supporting this are conflicting and indicate that some nutrients may be affected while others are not. Watson, Muir and Davidson (10) found that the level of feeding affected the utilization of rations of roughage alone differently than mixed rations.

The limitations of the present empirical determination of crude fiber were discussed by Crampton and Maynard (11) and they proposed the division of the carbohydrate fraction of feedstuffs into lignin, cellulose and other carbohydrates. Horwitt, Cowgill and Mendel (12) reviewed the long recognized faults of the ether extract method for the determination of fat and developed a relatively rapid method for the determination of true fat. They also investigated the value of an enzymatic method for the determination of crude fiber. There is a great need for an extension of the application of these and similar methods to feed analysis in order to determine their adequacy in the estimation of the feeding value of various roughages.

The present study was undertaken (a) to develop a method for evaluating quantitative digestion in the rumen, (b) to observe the effect of the plane of nutrition on digestion coefficients and rumen fill, and (c) to obtain information on the value of determining lignin, cellulose, crude fiber by an enzymatic method, and true fat in alfalfa hay.

#### METHODS

Six Holstein cows, five normal and one with rumen fistula, received alfalfa hay as the only feed in amounts of 10, 20 or 30 pounds per day. The rumen ingesta was removed from the cow with the fistula following a preliminary feeding period of at least 12 days at each level of feeding. The rumen contents were removed in the morning just before feeding (14 hours after the previous feeding) on six occasions (twice for each level of feeding), weighed, mixed, sampled for chemical analysis and replaced in the rumen. On one occasion the rumen contents were removed 24 hours after feeding rather than at the usual 14-hour period. The cow was weighed just before and after the rumen was emptied in order to check the weight of the material removed. A sample of feces was collected with each sample of rumen contents. Eleven digestion trials were conducted to supplement the results obtained with the rumen fistula cow. Three trials were at the 30-pound level, six at the 20-pound level and two at the 10-pound level. Each trial consisted of a 10-day preliminary period followed by 10 days of collection.

The chemical analyses which were made on the hay, rumen contents, and feces were moisture, protein, ash, ether extract, crude fiber, nitrogen-free extract and iron by the A. O. A. C. methods (13). Lignin, cellulose and other carbohydrates (by difference) were determined by the methods employed by Crampton and Maynard (11). Crude fiber (later referred to as "new crude fiber"), "new nitrogen-free extract" (by difference) and true fat were determined by the methods of Horwitt, Cowgill and Mendel (12). The cellulose determination was slightly modified by using a 50 ml. pyrex ignition tube for both digesting and centrifuging the sample, thereby eliminating the necessity of transferring the material from a 150 ml. round-bottom flask to a centrifuge tube. A temperature of 39° C. was used to incubate the lignin and new crude fiber samples. Linen (Butcher's) filtering cloth was used for filtering the new crude fiber sample because the solution passed through the linen much more rapidly than through various crucibles. For the final filtration, the linen and a beaker were weighed previously.

Rumen and total digestion coefficients were calculated by using (a) iron ratios and (b) lignin ratios. In addition, total digestion coefficients were calculated from the digestion trials. Bergeim (14) proposed a simplified method for the determination of food digestibility and utilization based on iron ratios. Iron oxide was added to the food and from the ratio of the amount of a given nutrient to the amount of iron in the food and feces, the percentage utilization was calculated. Dividing the fecal ratio by the food ratio and multiplying by 100 gave the percentage of the nutrient not digested. This percentage subtracted from 100 gave the percentage digested. Lignin was used in this investigation because of its highly inert character and the relatively large amount that is present in roughages. Knott and co-workers (15) illustrated the method of computation in the following formula:

$$100 - \left( \frac{\text{per cent nutrient in feces}}{\text{per cent iron in feces}} \times \frac{\text{per cent iron in feed}}{\text{per cent nutrient in feed}} \right) 100 = \text{digestibility}$$

In this investigation *rumen* was substituted for *feces* to calculate rumen digestion coefficients and *lignin* was substituted for *iron* when the lignin ratio was being used.

A Beckman pH meter was used to obtain the pH values of the rumen contents at regular intervals during the day on the 10-, 20- and 30-pound levels. Values on a ration of hay, silage and concentrate were also recorded. Rumen and rectal temperature readings were taken with the pH readings.

Barrel circumferences and body weights were recorded for various animals in the experimental herd receiving different levels of alfalfa hay or green alfalfa. These data were usually taken every third day just before feeding in the afternoon.

TABLE I  
*Composition of alfalfa hay, rumen contents and feces on the dry basis*

Nutrient	Alfalfa hay			Ruminant contents			Feces		
	30-lb. level	20-lb. level	10-lb. level	30-lb. level	20-lb. level	10-lb. level	30-lb. level	20-lb. level	10-lb. level
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Protein .....	16.2	18.1	16.3	13.5	14.8	11.1	11.7	12.5	10.3
Ash .....	6.8	7.4	7.0	9.5	9.9	9.4	10.9	9.7	9.3
Ether extract .....	2.6	2.2	1.7	2.9	2.5	2.3	4.4	4.1	2.2
True fat .....	0.51	0.69	0.67	1.09	0.83	0.80	0.47	0.34	0.53
Crude fiber .....	35.3	31.3	36.3	48.1	45.3	51.6	43.1	37.4	47.8
N-free extract .....	39.2	41.1	38.7	26.1	27.5	25.7	29.9	36.3	30.5
Lignin .....	15.7	15.8	17.7	29.1	30.8	33.2	31.1	29.4	32.3
Cellulose .....	36.3	29.2	34.2	36.2	34.6	39.5	33.3	28.9	36.4
Other carbohydrates .....	22.5	27.3	23.2	8.6	7.4	6.1	8.7	15.5	8.1
New crude fiber .....	53.2	48.1	53.9	73.6	71.8	81.9	73.6	67.9	73.9
New N-free extract .....	21.3	24.3	21.1	0.5	1.0	-3.2	-0.5	5.8	2.9
Iron .....	0.021	0.014	0.012	0.028	0.033	0.023	0.053	0.052	0.036

## RESULTS

Analyses of the alfalfa hay, rumen contents and feces at the various levels of hay consumption are given in table 1. It will be observed that the combined values for lignin and cellulose approach the value for new crude fiber and that the values for true fat vary independently of variations in ether extract. The biological value of these determinations is readily observed by referring to table 3 and noting the greater digestibility of other carbohydrates and new nitrogen-free extract in comparison to the nitrogen-free extract. The lower digestibility of new crude fiber compared with crude fiber and the characteristic digestibility of the lignin and cellulose fractions may also be seen. True fat was much more highly digestible than ether extract.

TABLE 2  
*Rumen and total digestion coefficients on the basis of iron and lignin ratios*

Nutrient	Coefficients of rumen digestion			Coefficients of total digestion		
	30-lb. level	20-lb. level	10-lb. level	30-lb. level	20-lb. level	10-lb. level
Iron ratios						
Dry matter . . . . .	25.0	57.6	47.9	60.4	73.1	66.7
Protein . . . . .	38.3	65.7	64.6	71.9	81.2	79.4
Ether extract . . . . .	15.7	48.2	25.7	32.4	49.1	53.4
True fat . . . . .	- 58.9	46.3	37.3	64.0	89.7	74.9
Crude fiber . . . . .	- 0.9	39.2	25.8	51.7	66.9	56.4
N-free extract . . . . .	51.0	71.9	65.3	69.9	76.1	74.2
Lignin . . . . .	- 41.6	18.5	1.9	21.9	49.1	40.2
Cellulose . . . . .	25.5	49.6	39.3	63.6	73.1	65.1
Other carbohydrates . . . . .	70.9	88.3	85.7	80.6	84.7	88.7
New crude fiber . . . . .	- 2.6	37.2	20.3	45.3	61.7	55.2
New N-free extract	97.6	94.2	100.0	99.1	92.5	91.2
Lignin ratios						
Dry matter . . . . .	47.5	48.7	46.7	49.5	46.3	45.2
Protein . . . . .	55.5	57.8	63.8	63.3	62.8	65.5
Ether extract . . . . .	40.0	35.1	24.5	13.2	- 1.8	20.8
True fat . . . . .	- 13.8	33.2	34.8	53.4	71.2	57.9
Crude fiber . . . . .	28.2	25.1	23.7	38.1	35.7	27.3
N-free extract . . . . .	64.9	65.5	64.5	61.4	52.3	56.8
Cellulose . . . . .	47.4	38.3	38.1	53.2	46.3	41.6
Other carbohydrates . . . . .	78.0	85.4	84.9	80.3	69.4	81.1
New crude fiber . . . . .	27.1	22.8	18.7	29.8	22.7	25.3
New N-free extract . . . . .	98.0	93.3	100.0	98.9	86.3	84.9
Iron . . . . .	27.5	- 22.8	- 2.6	- 27.5	- 100.0	- 64.2

Table 2 presents the rumen and total digestion coefficients calculated on the basis of lignin and iron ratios. Most of the coefficients obtained for rumen digestion by means of lignin ratios closely approach the actual values for total digestion given in table 3. Although the calculated total digestion coefficients at the 10-pound level compare favorably with the actual coefficients for that level, the calculated values for the other two levels are con-

TABLE 3  
*The relation of rumen digestion to total digestion and the effect of the level of feeding*

Nutrient	Rumen digestion, lignin ratio			Total apparent digestion*			Percentage of total occurring in rumen
	30-lb. level	20-lb. level	10-lb. level	30-lb. level	20-lb. level	10-lb. level	
Dry matter	45.7	48.7	46.7	56.3	55.8	46.9	84.4
Protein	55.5	57.8	63.8	68.9	68.4	64.7	80.6
Ether extract	-	40.0	35.1	24.5	17.2	13.4	-18.6
True fat	-	-13.8	33.2	34.8	65.3	72.5	-48.0
Crude fiber	-	28.2	25.1	23.7	45.6	44.9	36.2
N-free extract	64.9	65.5	64.5	60.1	66.6	56.9	108.0
Lignin	-	0.0†	0.0†	0.0†	17.0	15.4	-0.2
Cellulose	47.4	38.3	38.1	50.9	51.4	44.1	93.3
Other carbohydrates	78.0	85.4	84.9	85.0	84.7	80.2	91.8
New crude fiber	27.1	22.8	18.7	39.5	37.1	32.9	68.7
New N-free extract	98.0	93.3	100.0	86.6	90.0	72.8	113.2
Iron	-	27.5	-22.8	-2.6	-21.6	-68.6	-72.5

\* Determined by digestion trials.

† Lignin was assumed to be indigestible to facilitate calculations.

siderably lower than the actual coefficients. Calculations of rumen digestion with iron ratios gave highly variable results which were unusually low at the 30-pound level and somewhat high at the 20-pound level. The unusually high coefficients of total digestion obtained by using the iron index are explained by the negative iron balances in table 3.

The data presented in table 3 show the digestion coefficients of the various nutrients in the rumen on the basis of the lignin ratio, the total digestion coefficients obtained by digestion trials and the percentage of the total digestion that occurred in the rumen. The more simple carbohydrates represented by nitrogen-free extract, new nitrogen-free extract and other carbohydrates disappeared rapidly from the rumen. Digestible cellulose was largely removed while the feed was in the rumen while digestible crude fiber and new crude fiber were less than two-thirds removed before passage of the ingesta from the rumen. Of particular note among coefficients of rumen digestion is the negative coefficient of -13.8 per cent for true fat at the 30-pound level. This average value included one coefficient as low as -26.4 per cent.

The effect of the level of feeding on rumen and total digestion coefficients is shown in table 3. Increased hay consumption resulted in a decreased rumen digestion of protein and true fat and an increased digestion of ether extract, crude fiber and new crude fiber. Other nutrients did not vary directly with the level of feeding. Total digestion coefficients did not vary

TABLE 4

*Effect of the level of feeding on rumen fill, live weight, and barrel circumference*

Dry matter intake	Weight of rumen contents	Dry matter	Total dry matter	Weight of animal	Rumen fill as per cent of live weight	Barrel circumference
kg.	kg.	%	kg.	kg.	%	cm.
11.9	61.7	14.3	8.8	367	16.8	198
8.2	62.1	15.6	9.7	377	16.5	198
4.1	63.1	16.0	10.1	376	16.8	197
12.2	62.1	13.7	8.5	406	15.3	208
7.7	51.3	12.9	6.6	404	12.7	204
4.0	59.9	14.2	8.5	383	15.6	193
4.0	29.3*	14.5	4.2	368	8.0	191

\* Rumen contents removed 24 hours after feeding.

appreciably when the level of feeding was increased from 20 to 30 pounds. The change resulted in a slightly lower digestibility of nitrogen-free extract, new nitrogen-free extract and true fat and an increased digestibility of ether extract and new crude fiber. All constituents were less digestible at the 10-pound level than at the higher levels.

The data presented in table 4 show that the amount and the character of rumen fill remained fairly constant regardless of the amount of hay consumed. The data in tables 4 and 5 illustrate that the body weight and

TABLE 5

*Effect of the level of feeding alfalfa hay and green alfalfa on barrel circumference and live weight*

Cow no.	D12	A10	D11
Days fed .....	21 33 27 24	21 33 27 24	15 18 15
Lb. of hay .....	30 15 30 15	9 40 20 40	5 20 30
Barrel cir.* .....	248 239 243 234	222 227 225 233	206 214 225
Live weight† .....			978 1052 1139
Cow no.	A4	266	264
Days fed .....	6 21 9	6 21 9	12 21
Lb. of hay .....	40 20 40	30 ..... 30	30 15
Lb. of alfalfa‡ .....	100 .....	100 .....	.....
Barrel cir.* .....	240 231 240	218 212 219	241 232
Live weight† .....	1275 1222 1267	995 915 972	1153 1091

\* Circumference in centimeters.

† Weight in pounds.

‡ Green alfalfa.

barrel circumference increase or decrease directly with variations in dry matter intake. The failure of these values to drop when the dry matter intake of the rumen fistula cow decreased from 11.9 to 4.1 kg. was the only exception. Feeding green alfalfa, however, resulted in distinctly lower values for both live weight and barrel size, although the dry matter intake remained the same.

In figure 1 the diurnal variations in rumen pH at the various levels of feeding are illustrated graphically. There was a steady decline in rumen pH during the first six hours after feeding. After that time, the pH rose

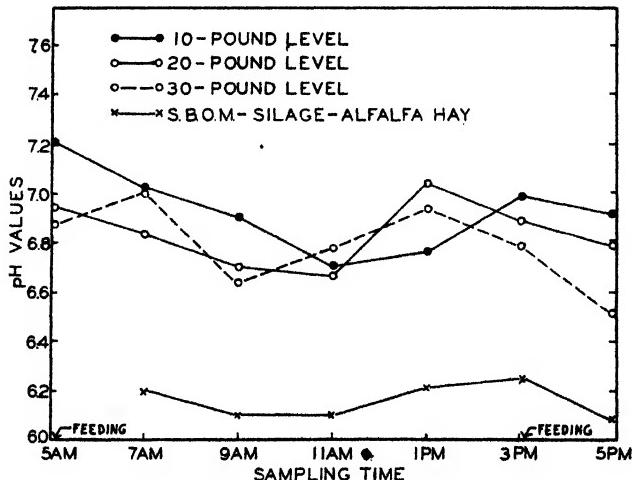


FIG. 1. Diurnal variations in the pH of rumen ingesta at various levels of alfalfa hay consumption.

until a maximum was reached just before feeding in the afternoon. This suggests a decrease in fermentation activities in the rumen about six hours following feeding. Rumen pH values on the alfalfa hay ration averaged 6.82 and were definitely higher than the pH values obtained on a ration of hay, silage and soybean oil meal. Variations of pH in different regions of the rumen at any one time never exceeded pH 0.3. Generally, the posterior region was more alkaline and the reticulum averaged 0.13 pH more alkaline than the rumen. These findings compare closely with the results reported by Monroe and Perkins (16). Rumen temperature was not affected by time after feeding or the level of hay consumption, although the highest values were obtained at the 30-pound level. The values ranged from 36.0 to 39.6° C. (mean 38.8° C.). If the few low values which resulted from water consumption are excluded, the mean rumen temperature was found to be 39° C. This value was 0.7° C. higher than the average rectal temperature. The difference was probably due to the more active fermentation in the rumen.

#### DISCUSSION

*Value of iron and lignin ratios in determining digestion coefficients.* Bergeim (14) reported that the iron ratio was a satisfactory method of determining food digestion and utilization in certain species but Knott and associates (15) reported that the iron ratio was not adaptable to digestion studies in the ruminant. Rathnow (23) used the iron index as a measure of digestion in the rumen and concluded that it served as a good indication of the rate of digestion. By this method he estimated that in three hours 32.1 per cent, in six hours 48.5 per cent and in nine hours 58.8 per cent of the dry matter in the rumen was digested. The data in tables 2 and 3 demonstrate the unreliability of the iron ratio as a measure of rumen digestion because the iron accumulated in the rumen in some instances and in others it passed from the rumen ahead of the ingesta.

The accuracy of the lignin index as a measure of rumen digestion is dependent upon the degree to which lignin is digested in the rumen. Any appreciable digestion of this substance in the rumen would result in low values for rumen digestion. The fact that the calculated coefficients for rumen digestion are high and in many instances approach or equal the total digestion coefficients indicate that lignin was not digested to any significant extent in the rumen and is, therefore, a suitable measure for rumen digestion. A comparison of the digestion coefficients for cellulose and new crude fiber further indicate that lignin is primarily digested after leaving the rumen. Digestible cellulose was largely removed while the ingesta was in the rumen but only about two-thirds of the digestible crude fiber was digested. The remaining one-third of the digestible crude fiber which was digested after leaving the rumen must have been lignin because most of the other major crude fiber fraction (cellulose) had been previously digested.

It is important to have some idea of the type and degree of error that would occur in event lignin were materially digested in the rumen. Calculations based on a comparison of cellulose and new crude fiber coefficients indicate that when the highest lignin digestibility coefficient was used (23.7 per cent) only 10 per cent of the lignin was digested in the rumen. By assuming a 10 per cent digestibility of lignin in the rumen, it was found that the percentage error in rumen digestion coefficients of highly digestible materials such as nitrogen-free extract, other carbohydrates and new nitrogen-free extract, would be nil and the coefficients for protein, ether extract and true fat would only be a few per cent. The percentage error for fibrous materials would be considerable with cellulose digestion coefficients being more nearly correct than those for the crude fiber fractions. These data indicate that the lignin ratio is a useful tool in ascertaining the digestibility of various nutrients in the rumen. Any errors that might occur would be negligible with the exception of coefficients for fibrous materials and in this instance the error would always be in the same direction, underestimation, and could be allowed for. This method of studying rumen activity should fill a need for some quantitative measure of rumen digestion and should be of considerable value in affording an understanding of the degree and significance of rumen digestion compared with the subsequent digestion that occurs during the passage of the ingesta through the remainder of the digestive tract.

*Quantitative digestion in the rumen.* It should be borne in mind that the digestion coefficients obtained by calculations with lignin ratios do not necessarily imply that the nutrients are in an absorbable state. Literally, they indicate the percentages of the various nutrients that pass from the rumen ahead of the lignin. In the case of cellulose and crude fiber the coefficients probably indicate completed digestion, whereas the more simple carbohydrates would probably be completely broken down and in solution. Other nutrients would seem to be sufficiently decomposed to allow digestion to be completed rapidly in the remaining portions of the digestive tract.

The carbohydrate materials included in the nitrogen-free extract, other carbohydrates and new nitrogen-free extract passed rapidly from the rumen and in most instances 100 per cent of the digestible portion of these materials had disappeared from the rumen by the time of sampling. It is probable that these more simple carbohydrates did not merely enter solution but that some were fermented by rumen bacteria before their passage to the other stomach compartments.

Digestible cellulose was largely removed by the fermentation processes of rumen micro-organisms with an average of 93.3 per cent being digested in the rumen at the 30-pound level of feeding and an average of 85.0 per cent being digested for all levels. Lignin was not digested to any material extent while in the rumen but was digested in variable amounts in the

remaining portions of the digestive tract. Csonka and co-workers (17) concluded from *in vitro* experiments that the degradation of lignin occurs in the stomach compartments of the cow and is not brought about by bacteria but possibly by some enzyme of the gastric juice. About two-thirds of the digestible crude fiber fractions and 90 per cent of the digestible dry matter were digested in the rumen.

Rumen digestion coefficients for ether extract were higher than total digestion coefficients indicating an accumulation of ether extractive material after passage of the ingesta from the rumen. That this material was non-fat in character is indicated by the fact that even though true fat was readily digested, a negative coefficient for ether extract might occur.

Rumen digestion coefficients for true fat indicate the probable products of the rumen digestion of cellulose and other carbohydrate materials. At the 30-pound level, at which bacterial activity would be greatest, there was an average increase of 13.8 per cent true fat in the rumen and one of the individual values included in this average represented a 26.4 per cent increase. Only two out of seven rumen digestion coefficients for true fat exceeded 20 per cent, while the total digestion of true fat averaged 66.1 per cent. Failure to find an increase in fat at the two lower levels may have been due to the continual passage of the products from the rumen and also to the fact that the samples were taken after the height of digestion had passed. These findings indicate the synthesis of fat by rumen micro-organisms.

Supporting a synthesis of fat in the rumen are *in vitro* studies of cellulose fermentation by rumen bacteria by Woodman and Evans (5) and Pochon (6). Nicholson and Shearer (18) observed much volatile fatty acid in the rumen of animals studied at post mortem. Ritzman and Benedict (19) in discussing the metabolic stimulus of food stated, "in our judgment this average increase of 30 per cent noted with sheep confirms the striking increase noted with steers on a protein-poor, carbohydrate-rich maintenance ration, and suggests again the significance of the old view of Grouven that the path of absorption of carbohydrates in the ruminant is through the fermentative processes. These processes result in flooding the body with a large amount of the lower fatty acids which stimulate metabolism." Quittek (3) suggested the synthesis of fat in the rumen but his methods were not adequate enough to allow a definite conclusion and Krzywanek and Quittek (4) later concluded their results did not indicate a fat synthesis. The production of fatty acids from cellulose fermentation *in vitro* and the data supporting the formation of fat in the rumen suggest the possibility that the products of rumen digestion may play an important role in the physiology of ketosis in cattle. This subject has been discussed by Duncan and associates (20).

When the removal of the rumen contents was delayed until 24 hours after feeding, only a slight increase in rumen digestion coefficients was observed. This indicates that rumen digestion of the alfalfa hay was practically completed prior to 14 hours after feeding.

*Effect of the level of feeding on rumen fill and digestion coefficients.* The amount and character of the rumen fill remained fairly constant in spite of variations in hay consumption as great as 10 to 30 pounds. This finding is in agreement with the data of Ewing and Wright (21) who found that the weight and the dry matter content in the rumen of steers receiving from 1.66 to 3.84 kg. of dry matter daily did not differ significantly. These data indicate that the rumen fill was maintained at a constant level even though an increase in the level of feeding was always followed by an increase in barrel circumference and live weight. The change in barrel circumference was marked but was not perceptible to the eye. Feeding green alfalfa resulted in a decrease in body weight and barrel size even though the dry matter intake remained the same.

As the level of feeding increased there was a decrease in the digestion of protein and true fat in the rumen and an increase in the digestion of ether extract, crude fiber and new crude fiber. The rumen seems capable of handling highly variable quantities of roughage with equal efficiency. Total digestion coefficients for dry matter, protein, crude fiber, cellulose, lignin and other carbohydrates were not affected by increasing the level of feeding from 20 to 30 pounds of hay per day, while new crude fiber and ether extract were more digestible and only nitrogen-free extract, new nitrogen-free extract and true fat were less digestible. These results tend to support the conclusion of Watson and associates (10) that the plane of nutrition does not have any marked effect upon the digestibility of dried roughages. The marked decrease in digestibility which is often associated with a high plane of nutrition was not observed in this study. The low digestibility of all nutrients at the 10-pound level was attributed to the sub-maintenance level of nutrition. Watson and associates (10) and Forbes and co-workers (22) also observed a lowered digestibility on sub-maintenance rations.

*Value of lignin and cellulose, new crude fiber and true fat in feed analysis.* Partitioning the carbohydrate fraction of alfalfa hay into lignin, cellulose and other carbohydrates was found to be of greater value in estimating the biological value of hay than the usual division into crude fiber and nitrogen-free extract. Other carbohydrates averaged 21 per cent more digestible than the nitrogen-free extract, cellulose was 50.0 per cent digestible and lignin varied in digestibility from -5.1 to 23.7 per cent.

The values obtained by the enzymatic method for crude fiber gave a much sharper distinction between the highly digestible and poorly digestible fractions of carbohydrate than the values obtained by the common crude

fiber method. By the common method the difference in the digestibility of crude fiber and nitrogen-free extract was only 19.5 per cent compared with 49 per cent by the enzymatic method. Practically 100 per cent of the new nitrogen-free extract disappeared in the rumen compared with 65 per cent of the nitrogen-free extract and 83 per cent of other carbohydrates. The new nitrogen-free extract was slightly more digestible than other carbohydrates indicating that the material—other than cellulose or lignin—present in the new crude fiber fraction was poorly digestible in character. The enzymatic method for crude fiber requires somewhat less labor than the lignin and cellulose determinations but more time is required to obtain the results because an 8-day incubation period is necessary in comparison to a 12-hour incubation period for lignin.

The true fat values indicate greater significance than the ether extract values. True fats were found to be 66.1 per cent digestible, while only 8.6 per cent of the ether extract was digestible. Attention should be directed to the fact that the methods generally employed for the determination of fat in feed materials do not completely remove the fat and allied substances. In view of the low values obtained in this study for true fat, it is suggested that additional methods should be investigated for the complete extraction and determination of fat in feeding stuffs.

#### SUMMARY AND CONCLUSIONS

One rumen fistula and five normal Holstein cows received alfalfa hay in amounts of 10, 20, or 30 pounds per day. Rumen contents were removed 14 hours after feeding on six occasions and 24 hours after feeding on one occasion. A total of 11 digestion trials were conducted. The chemical analyses which were made on the hay, rumen contents and feces were moisture, protein, ash, ether extract, true fat, crude fiber, nitrogen-free extract, lignin, cellulose, other carbohydrates (by difference), crude fiber by an enzymatic method, new nitrogen-free extract (by difference), and iron.

The use of lignin ratios is proposed as a method of studying quantitative digestion in the rumen. This method is useful in studying the significance of rumen digestion as related to subsequent digestion in the remainder of the digestive tract and is relatively accurate. Iron ratios are not reliable for determining rumen or total digestion coefficients.

An average of 90 per cent of the digestible dry matter passed from the rumen before 14 hours after feeding. Digestible nitrogen-free extract, other carbohydrates and new nitrogen-free extract were completely removed from the ingesta during rumen digestion. Eighty-five per cent of the digestible cellulose had been digested at the time of sampling. Lignin did not appear to be appreciably digested in the rumen even though total digestion ranged up to 23.7 per cent but digestible crude fiber was two-thirds digested

in the rumen. There was an accumulation of ether extractive substances after the ingesta passed from the rumen.

True fat was not digested to any extent in the rumen and at the 30-pound level of feeding there was an actual increase in true fat. These findings suggest the synthesis of fat in the rumen by micro-organisms from such materials as cellulose and other carbohydrates.

Rumen pH on a ration of alfalfa hay was neutral or slightly acid (average pH 6.82) and the maximum acidity was reached about six hours after feeding. A mixed ration gave lower rumen pH values. Rumen temperature approximated 39° C.

The character and amount of rumen fill were not affected by the amount of feed consumed, although the body weight and barrel circumference varied directly with hay consumption.

As the plane of nutrition increased the rumen digestion of protein and true fat decreased while that of ether extract, crude fiber and new crude fiber increased. Total digestion coefficients for nitrogen-free extract, new nitrogen-free extract and true fat decreased as the level of feeding increased from 20 to 30 pounds. The other nutrients were either not affected or increased slightly in digestibility. The low digestibility of all nutrients at the 10-pound level was attributed to the sub-maintenance level of nutrition.

The separation of the carbohydrate fraction into lignin, cellulose and other carbohydrates is a better index to the biological value of alfalfa hay than the present division into crude fiber and nitrogen-free extract. The values obtained by the enzymatic determination of crude fiber gave a much sharper biological distinction between the two carbohydrate fractions than the standard method. Inasmuch as true fat averaged 66.1 per cent digestible and ether extract only 8.6 per cent, it is evident that the true fat determination is of greater biological significance than the ether extract determination.

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## A RAPID COLORIMETRIC METHOD FOR THE DETERMINATION OF LACTIC ACID IN MILK

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Many methods for determining lactic acid in biological fluids have been reported. Several have been applied successfully to dairy products. Most of these methods, however, are too time consuming to be used regularly in the control laboratory. A search was made, therefore, for a method which was both rapid and reliable: A method using a simple procedure for the removal of interfering substances; a simple oxidation procedure; and a simple means of estimating the quantity of lactic acid present.

The method Mendel and Goldscheider (5) used for determining the lactic acid in blood seemed to be the most promising, but when applied to milk gave unreliable results. Many variations were tried, one of which is reported below. The method for removing interfering substances is almost identical with that described by Troy and Sharp (7). Their work on precipitation procedures was repeated to determine the most satisfactory technique to be used in conjunction with the colorimetric method, but no change was found necessary.

### PROCEDURE

Weigh 5 grams of milk in a 50 ml. volumetric flask. Add 30 ml. of cold water and mix. Dilute a portion of a 25 per cent solution of copper sulphate 1:3 and add dropwise with agitation 2-8 drops of the dilute solution to the water and milk mixture until precipitation occurs. Warm the flask in hot water until the contents reach 45° C. Add 6 ml. of 25 per cent copper sulphate solution and mix thoroughly by rotation. Hold the contents of the flask at 45° to 47° C. for 8 to 10 minutes. Next loosen any curd particles which may adhere to the flask with a bent wire. Add 6 ml. of calcium hydroxide suspension. This suspension is prepared by slaking 300 grams of the best quality calcium oxide using 1400 ml. water and working through a 24 mesh screen after 30 minutes standing. Before mixing the lime with the other constituents in the flask, water should be added to bring the contents to exactly 50 ml. Then mix until the contents are homogeneous and allow to stand at 45° to 47° C. for 10 minutes. Cool to 25° C. and filter, discarding the first few ml. of filtrate.

Place 1.0 ml. of the filtrate in a thoroughly clean and dry test tube. Hold the test tube in ice water and allow 5.0 ml. of cold concentrated sulphuric acid (arsenic free) to run down the side wall of the test tube. Mix gently while cooling. Place mixture in boiling water for exactly 5 minutes.

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Cool in ice water for three. Add 4 drops of a 0.1 per cent solution of veratrole in water and mix. Hold in ice water for 60 minutes and compare colors with artificial or natural standards.

Natural standards may be prepared according to Hillig (3). Dissolve 106.6 mg. of purified lithium lactate in 100 ml. of distilled water. 1.0 ml. of this solution contains the equivalent of 0.1 per cent lactic acid. Consequently, to prepare a 0.01 lactic acid standard, 5.00 ml. of this solution is diluted to exactly 50.0 ml. and mixed. One ml. of this dilution is then placed in a test tube and 5 ml. of concentrated sulphuric acid added. The mixture is held in boiling water 5 minutes and cooled in ice water 3 minutes. Four drops of a 0.1 per cent solution of veratrole is then added and the color observed after 60 minutes. Additional standards may be made by diluting the original lithium lactate solution as required.

Artificial standards which are fairly satisfactory may be prepared according to the method of Nordbö (6). Mix 25.0 ml. of a 0.01 per cent solution of fuchsin in water and 4.75 ml. of a 0.01 per cent solution of Tropaolin 000. (The mixture must be diluted immediately for a precipitate forms on standing.) One tenth ml. of this mixture diluted to 50.0 ml. of solution is equivalent to approximately 0.005 per cent lactic acid; 0.25 ml. diluted to 50 ml. is equivalent to approximately 0.01 per cent and 0.9 ml. diluted to 50 ml. is equivalent to 0.02 per cent. Nordbö's standards are only suitable for concentrations below 0.03 per cent. Above this percentage, the yellow color increases in intensity. Consequently, for a 0.05 per cent lactic acid standard, it is necessary to add 0.4 ml. of .01 per cent Tropaolin and 1.8 ml. of .01 per cent fuchsin to 47.8 ml. of water; for a 0.07 per cent standard, 1.0 ml. of Tropaolin and 2.0 ml. of fuchsin to 47.0 ml. of water; and for a 0.10 per cent standard, 2.0 ml. of Tropaolin and 2.0 ml. of fuchsin to 46.0 ml. of water. These artificial standards should be checked against natural standards before using.

#### DISCUSSION

According to the authors, the original test which was applied to blood is accurate to  $\pm 0.004$  per cent between 0.001 and 0.05 per cent (5) and 1 part in 400,000 lactic acid could be detected. Mendel (4) later made two improvements which he stated made possible the detection of one part in 1,000,000. However, when applied to milk, the original test was sensitive to 0.01 per cent and had a limited degree of accuracy ( $\pm 0.04$  per cent). With the modification outlined above, the test is sensitive to 0.005 per cent and accurate to  $\pm 0.004$  per cent between the ranges of 0.005 and 0.12 per cent lactic acid providing the procedure is closely followed.

Mendel (4) has pointed out that small changes in the concentration of the sulphuric acid may lead to inaccurate results. A difference of 2 to 3 per cent in the concentration may result in as much as a 15 to 20 per cent

error. It is wise, therefore, to check frequently the color of the artificial standards. In this respect, it should be noted that the individual analyst should try several different quantities of sulphuric acid (*i.e.*, 4.5, 4.75, 5.0 ml. etc.,) in the preparation of natural standards in order to ascertain the exact amount to add to the 1.0 ml. filtrate to obtain the greatest degree of color development for each batch of sulphuric acid. Derviz (1) describes a method for purifying and storing sulphuric acid so as to maintain a constant concentration.

Mendel (4) makes the recommendation that after the mixture of acid and filtrate has been held in boiling water five minutes, it be cooled 2 minutes in ice water, veratrole added, and set at 25° C. After 20 minutes, the color may be compared with standards similarly treated. This method may be employed whenever results of great accuracy are not desired.

Mendel and Goldscheider (5) used 0.1 ml. of a 0.125 per cent veratrole in 99.8 per cent alcohol. Other investigators suggested 0.05 ml. of a 1:800 solution in 96 per cent alcohol (6), 0.1 ml. of 20 per cent veratrole solution in glacial acetic acid (1) and 0.1 ml. of 20 per cent in absolute alcohol (2). These were all tried, but none gave as satisfactory results as 4 drops of a 0.1 per cent solution in water. Various solutions of guaiacol, hydroquinone, and Schiff's reagent were also tried and considered unsatisfactory.

Although the original method called for a 4 minute heating period, 0.5 ml. filtrate and 3.0 ml. of conc. H<sub>2</sub>SO<sub>4</sub>, it was found that 5 minute heating period, 1.0 ml. filtrate and 5.0 ml. of concentrated sulphuric acid gave more uniform results.

The same substances interfere with this method as those Troy and Sharp (7) found to interfere with theirs. Formaldehyde, overneutralization, rancidity, sucrose, and products resulting from the heating of milk at or near the boiling point for one half hour or longer resulted in high values. Mendel and Goldscheider state, however, that acetone, β-oxybutyric acid, acetic acid, urea, uric acid, creatin, creatinin, glycocol, alanine, and propionic acid, do not interfere with the color development.

Traces of organic matter interfere with proper color development and consequently the test tubes must be thoroughly clean and kept stoppered as much as possible during the determination.

When greater rapidity of testing is required, the precipitate of proteins, lactose and fat, may be centrifuged. In this case, cream test bottles (18 gram body, 9 gram neck, graduated in 1 per cent between 0 and 55 per cent) were selected from stock and graduated to contain 50.0 ml. at 20° C. The milk was weighed in these bottles which, after precipitation and cooling, were placed in a Babcock tester and centrifuged 4 minutes. The centrifugate was then decanted through a fluted filter, the first few ml. of filtrate being discarded.

## SUMMARY

A rapid colorimetric method for the quantitative estimation of lactic acid in milk is described. The steps involved are precipitation of fat, lactose and protein with copper sulphate and calcium hydroxide, filtering, adding concentrated sulphuric acid (arsenic free) to the filtrate, heating, cooling, and adding veratrole. A red color develops on standing which is in proportion to the amount of lactic acid present.

The test is sensitive to about 0.005 per cent and is accurate to  $\pm$  0.004 per cent between the ranges of 0.005 and 0.12 per cent lactic acid.

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# GROWTH OF CERTAIN ORGANISMS OF IMPORTANCE TO THE DAIRY INDUSTRY ON NEW STANDARD MILK AGAR\*

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The medium used for making counts on milk and other dairy products, which became official July 1, 1939, has created considerable interest as to the effect it will have on dairy control practices. This paper deals with the relative amount of growth of certain pure cultures of bacteria on the old and new standard agars together with the total number of colonies as determined with these two agars on milk samples collected aseptically from an experimental dairy herd. The animals in the herd were divided into three groups: Those known to be free of streptococcal mastitis; those suspected of the disease because of high leucocyte counts; and those with clinical evidence of the disease and with long chained streptococci in the incubated milk.

## RESULTS WITH PURE CULTURE STUDY

The pure cultures used were a series of pathogenic and non-pathogenic Gram-positive cocci and bacilli of importance to the dairy industry. These organisms were examined carefully for purity and identified. The "pyogenic" streptococci were all typical of the group as outlined by Sherman (1). The two cultures of *S. scarlatinae* (?) name were from clinically typical severe cases of scarlet fever and were of proved toxicity. The 53 cultures of *S. agalactiae* were isolated from chronic cases of bovine mastitis from twenty cows in one herd. These cultures had been isolated from one to nine months before being used. The seven, "Animal Pyogenes," streptococci were obtained from equine infectious processes. The pure cultures of non-pathogenic organisms were obtained from various workers in the field of dairy bacteriology. These cultures had been identified by others and were re-examined only to determine purity before being used in this study.

Sugar-free meat infusion agar slants were used as a medium for the cultivation of pure cultures used in this experiment. Within 24-hours after inoculation all cultures had grown enough to permit the preparation of saline suspensions which could be standardized as to turbidity. From these suspensions, serial dilutions were made and plated in duplicate. One set was poured with old standard agar (2) and the other with new standard agar (3). The plates were then incubated at 37° C. for 48 hours. Counts were made with: (a) the unaided eye; (b) Quebec colony counter, and (c) the 10× and 30× wide field binoculars.

The ability of the organisms to develop observable colonies on the two agars is clearly shown in table 1. In case of certain of the organisms, com-

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TABLE 1  
*Result of pure culture studies*

Organism	Strains	Colony detection on	
		New medium	Old medium
<i>S. scarlatinae</i>	2	Easily countable Quebec counter	None countable, 30× magnification
<i>S. agalactiae</i>	53	All easily countable Quebec counter	5—countable Quebec counter 20—10× magnification 10—30× magnification 18—none countable
“ <i>Animal Pyogenes</i> ” <i>Streptococci</i>	7	All easily countable Quebec counter	None countable Quebec counter
<i>S. durans</i>	1	Easily countable	Not countable Quebec counter
<i>S. lactis</i>	1	Easily countable Quebec counter	Not countable Quebec counter
<i>S. fecalis</i>	1	Easily countable Quebec counter	Not countable Quebec counter
<i>S. zymogenes</i>	1	Easily countable Quebec counter	Not countable Quebec counter
<i>L. bulgaricus</i>	4	1 countable 3 non-countable	None countable
<i>L. acidophilus</i>	2	Easily countable	1—not countable 1—difficultly countable
<i>S. thermophilus</i>	1	None countable	Not countable
<i>S. liquefaciens</i>	5	4 countable 1 not countable	4—pin-point not ordinarily countable 1—not countable
<i>L. casei</i>	3	1 countable 2 countable with difficulty	Not countable

plete inability to develop in the new medium is unquestionably due to lack of necessary nutrients. With other organisms, *S. agalactiae* for instance, ability to develop on the old medium apparently depends on strain variation. In all instances, with the exception of the culture of *S. thermophilus*, one or more strains of each grew well on the new medium. The addition of such nutrients as glucose, skim-milk and possibly tryptone makes a more suitable medium for the growth of these organisms.

#### RESULTS OF PLATE COUNTS ON MILK

The milk samples were obtained from the following groups of experimental cows: (a) 26 cows known to be free of mastitis; (b) 16 cows suspected of mastitis; (c) 26 cows known to have mastitis. All cows included in each group had been so classified for a period of at least three months and some had been grouped, as above described, for a period of 24 months. Equal quantities of milk, as far as practicable, were collected aseptically from each quarter and taken immediately to the laboratory for analysis.

A summary of the results on milk samples is given in table 2. From an examination of these data it is evident that the quality of the milk from the

TABLE 2  
*Milk sample studies*

Group of cows	Mastitis free		Mastitis suspects		Mastitis positive		
	Medium	New	Old	New	Old	New	Old
Number of animals in group		26	26	16	16	26	26
Log. Ave. count		126	47	912	389	4,470	2,690
Minimum count		3	1	50	30	300	10
Maximum count		17,500	12,000	4,800	4,700	896,000	634,000
Median count		77	35	1,145	665	4,350	2,200

three groups is quite different. The minimum, median and maximum counts on the three groups of cows has been determined. In all instances the minimum and maximum counts from the same animal were consistently higher in the new medium. The median count for each group of samples indicates that the new medium gives counts about double those obtained on the old medium.

In addition to the above described examples of the superiority of the new agar, it is of interest to compare the results obtained by using "new," "old" and meat infusion blood agars in a study of an epidemic of septic sore throat involving approximately forty cases. The total count per ml. milk from the involved quarter of the animal responsible for the epidemic as plated on the "old," "new" and blood agars was 5,800, 149,000, and 115,000, respectively. On blood agar 105,000 or 91 per cent of the total number of colonies counted were beta-hemolytic streptococci which were later found to belong to Lancefield group A.

#### CONCLUSIONS

It is clearly shown by this investigation that the pyogenic streptococci which were studied responded well to the new medium. The other streptococci and lactobacilli did not show the same degree of response; but growth was definitely improved in the case of most, but not all, of the organisms.

Milk from three groups of cows, in a mastitis control project, individually and collectively, showed definitely, higher plate counts when the new medium was used.

When the milk from an animal responsible for an outbreak of septic sore throat was plated on the new medium, there was a higher count than when blood agar was used. However, the use of blood agar is essential in the detection of such organisms and should be used in all such investigations.

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## A STUDY OF THE REDUCING SYSTEM OF MILK\*

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Since the introduction of the chemical method for the determination of ascorbic acid in biological materials, numerous studies have been conducted dealing with the ascorbic acid content of milk. However, to date, little attention has been given to the possibility that milk may contain other reducing substances, which, under certain conditions, may exert an influence similar to that exhibited by ascorbic acid. This study was conducted, therefore, with the view of determining the total reducing power of milk as measured by potassium iodate titration procedure, and then ascertaining the proportion of this power which is contributed by ascorbic acid.

Two reducing substances which are present in many biological systems and which have attracted considerable attention of research workers are ascorbic acid and glutathione. Fresh raw milk contains usually from 15 to 30 mg. of ascorbic acid per liter (5, 16, 11, 2), which exists in its entirety in the reduced form (9). Age, aeration, sunlight, and contamination with copper cause a destruction of the ascorbic acid in milk (16, 15, 14, 4), whereas heat, *per se*, exerts only a negligible effect upon the ascorbic acid content (2, 4).

The presence of glutathione in milk is disputed. Martini (10) reports that milk contains about 8 mg. per cent, but Jackson (6) Gould and Sommer (3) and Josephson and Doan (8) were unable to secure positive nitroprusside tests on normal raw milk. However, treatment of raw milk with sodium cyanide results in positive nitroprusside reactions (3, 6).

Jackson (7) observed that fresh, anaerobically drawn milk exhibited much greater reducing power than did the same milk if exposed for a few minutes to the atmosphere. Whether this indicates the presence of a strong reducing substance in milk which is quickly oxidized by atmospheric oxygen, or whether the change is merely due to the diffusion of oxygen into the milk has not been shown.

In addition to ascorbic acid, and probably glutathione or cysteine, the flavin system of milk is also of importance in connection with oxidation-reduction phenomena (4, 7). Jackson found that when whey concentrates were added to milk, the decoloration of methylene blue was greatly hastened.

### EXPERIMENTAL METHOD

The methods used were those suggested by the work of Woodward (17) and DeWitt and Sure (1). The  $KIO_3$  titration procedure of Woodward

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and Fry (18) was utilized to measure that power which may for convenience be called the "total reducing power" of the milk. The method is known to measure both the glutathione and the ascorbic acid content and has been used for that purpose (1, 17). The ascorbic acid was determined by the 2, 6-dichlorophenolindophenol titration procedure adapted to milk by Sharp (11).

The  $\text{KIO}_3$  method of Woodward and Fry (18) for glutathione in blood was modified only insofar as to permit the titration of a more concentrated filtrate. Forty milliliters of milk were diluted with 50 ml. of water, and the proteins precipitated with 10 ml. of molar sulfosalicylic acid solution. Ten milliliters of the filtrate were titrated with 0.001N  $\text{KIO}_3$  solution. The filtrate possessed a pH well under 2. Comparisons of this slightly modified procedure with the original micro procedure were made on fresh raw milk, and close agreement between the methods was secured.

The use of iodoacetate to block out the -SH groups was made, in general, on the basis of the work of Schultze, Stotz, and King (12).

Milk used for the study was in most instances obtained directly from the College herd. It was collected in such a manner as to eliminate contamination with copper. The heating of the milk was conducted in a glass flask suspended in a boiling water bath. Temperatures were controlled well within the limits of  $0.5^\circ \text{C}$ .

#### RESULTS

*Comparison of total reducing capacity with that contributed by ascorbic acid:* The titration of milk with the 0.001N solution of  $\text{KIO}_3$  gave values which were surprisingly high when calculated as glutathione or ascorbic acid. These results are presented in table 1, columns (1) and (2). These values range approximately from 119 to 192 when calculated as mg. of glutathione per liter, or from 34 to 55 when expressed as mg. of ascorbic acid per liter.

That the  $\text{KIO}_3$  values are decidedly higher than would be the case if the ascorbic acid alone were responsible may be seen by comparing columns numbered two and three. The actual ascorbic acid values, as determined by titration with 2, 6-dichlorophenolindophenol, range only from 11.8 to 21.9 mg. per liter, as contrasted to the approximate range of 34–55 mg. per liter obtained from the  $\text{KIO}_3$  titration. As shown in the final column, the ascorbic acid usually accounted for only 30–40 per cent of the total reducing action of the milk as determined with  $\text{KIO}_3$ , although in certain trials the ascorbic acid accounted for from 40 to 60 per cent of the total reducing power.

*Influence of sunlight and copper on the total reducing capacity:* Further evidence to show that the  $\text{KIO}_3$  titration values are due only partially to the ascorbic acid was obtained when fresh raw milk was exposed either to

TABLE 1

*Comparison of 2, 6-dichlorophenolindophenol and KIO<sub>3</sub> values when calculated as ascorbic acid and glutathione*

Sample	KIO <sub>3</sub> values		Actual ascorbic acid value*	Per cent actual ascorbic acid value of total KIO <sub>3</sub> value
	As GSH (1)	As ascorbic acid (2)		
mg./liter	mg./liter	mg./liter		
1	134.0	38.29	12.07	31.52
2	118.9	33.97	11.83	34.82
3	144.2	41.20	12.51	30.36
4	128.8	36.80	13.04	35.43
5	174.8	49.94	18.56	37.16
6	191.7	54.77	21.93	40.04
7	174.9	49.97	15.89	31.80
8	165.7	47.34	17.94	37.90
9	158.0	45.14	17.48	38.72
10	145.7	41.63	15.75	37.83
11	164.9	47.11	18.90	40.12
12	121.0	34.63	15.79	45.60
13	145.7	41.63	21.15	50.80
14	126.3	36.08	21.09	58.45

\* Determined with 2, 6-dichlorophenolindophenol.

sunlight for one hour or treated with 2 p.p.m. of copper for three hours. The KIO<sub>3</sub> values secured before and after such treatment and their comparison with the correct indophenol values are shown in table 2.

The KIO<sub>3</sub> results, when expressed either as glutathione or ascorbic acid show rather high values following the treatment of the milk with the sunlight or with the copper. Contrasted with this is the fact that these two agents, *i.e.*, sunlight or copper, destroy the ascorbic acid. In fact, they are utilized for this purpose in order to determine the so-called residual titer, or blank, in the indophenol titration. The ascorbic acid values shown in the table have been corrected for this residual titer.

The data in table 2 show further that the decrease in KIO<sub>3</sub> values caused by sunlight or copper is doubtless due entirely to the ascorbic acid itself, and not to any appreciable extent to the other reducing substances which are responsible for the remainder of the KIO<sub>3</sub> titer. This may be observed by comparing the columns numbered six and seven. The values in these columns show close agreement as indicated by the differences in column seven.

*Use of iodoacetate:* If the KIO<sub>3</sub> titer in milk is partly due to -SH linkages, iodoacetate should block out such groups and thus decrease the titer accordingly. Consequently, trials were conducted in which the milk was treated with sufficient iodoacetate to make a 0.1 molar concentration in the milk. Following the addition of the iodoacetate, the milk was stored at 4° C. for one hour. KIO<sub>3</sub> and indophenol titrations were made on the milk before and after treatment. The results of four trials are presented in table 3.

TABLE 2  
*The influence of sunlight and copper on the  $KIO_3$  and  $^2, 6$ -dichlorophenolindophenol values of milk\**

Treatment of sample	$KIO_3$ values						Actual ascorbic acid values (7)	Differ- ence (7) from (6)		
	As GSH			As ascorbic acid						
	Original (1)	After (2)	Difference (3)	Original (4)	After (5)	Difference (6)				
	mg./liter	mg./liter	mg./liter	mg./liter	mg./liter	mg./liter	mg./liter	mg./liter		
Sunlight	134.0	92.0	42.0	38.29	26.29	12.00	12.07	+ 0.07		
"	118.9	69.0	49.9	33.97	19.71	14.26	11.83	- 2.43		
"	128.8	69.0	59.8	36.80	19.71	17.09	13.04	- 4.05		
"	121.2	69.0	52.2	34.63	19.71	14.91	15.79	+ 0.88		
Copper	174.9	105.8	69.1	49.97	30.23	19.74	15.89	- 3.85		
"	165.7	109.7	56.0	47.34	31.34	16.00	17.94	+ 1.94		
"	158.0	92.8	65.2	45.14	26.51	18.63	17.48	- 1.15		
"	145.7	79.0	66.7	41.63	22.57	19.06	21.15	+ 2.09		
"	145.7	92.0	53.7	41.63	26.29	15.34	15.75	+ 0.41		
"	164.9	92.0	72.9	47.11	26.29	20.83	18.90	- 1.93		

\* Milk treated with bright sunlight for one hour or with 2 p.p.m. of copper for three hours.

TABLE 3  
*The influence of iodoacetate on the KIO<sub>3</sub> and 2, 6-dichlorophenolindophenol titration values of milk*

Trials	KIO <sub>3</sub> values as glutathione		2, 6-dichlorophenolindophenol values as ascorbic acid	
	Before treatment	After treatment	Before treatment	After treatment
1	mg./100 ml.	mg./100 ml.	mg./liter	mg./liter
2	13.43	10.20	11.38	12.74
3	15.43	10.58	17.35	17.51
4	14.95	11.50	15.52	15.52
	12.63	11.88	21.09	19.50

These data show that the iodoacetate treatment slightly lowered the KIO<sub>3</sub> values, whereas it had no definite effect upon the ascorbic acid content. The KIO<sub>3</sub> values decreased on the average, to an extent equivalent to approximately 3 mg. of glutathione per 100 ml. milk. Numerous other trials gave similar results.

*Use of zinc dust:* To determine the total glutathione content of blood, Woodward and Fry (17) added zinc dust to the sulfosalicylic acid filtrate. This procedure reduced the oxidized form of glutathione so that it could be measured by the KIO<sub>3</sub> titration. On this basis, the same procedure was utilized in this study to ascertain if the zinc dust treatment would increase the KIO<sub>3</sub> values of milk. Results of four trials are presented in table 4.

TABLE 4  
*The influence of zinc dust treatment on the KIO<sub>3</sub> values*

sample	KIO <sub>3</sub> values as glutathione	
	Before reduction	After reduction
	mg./100 ml.	mg./100 ml.
1	14.18	14.58
2	15.33	14.95
3	12.65	13.43
4	16.33	16.10

These results show the zinc dust to have no definite effect on the KIO<sub>3</sub> values. The values were substantially unaltered by the reduction, indicating that milk contains no substance in the oxidized form which may be reduced by the method and exposure time used in this experiment.

*Influence of heat on the total reducing capacity:* When milk has been heated sufficiently high the KIO<sub>3</sub> values are lowered, whereas the ascorbic acid content remains practically unchanged. This is illustrated by figure 1.

This graph shows that the KIO<sub>3</sub> values, (expressed as mg. glutathione per 100 ml.) undergo marked decreases in milk which has been heated to 80° C. or above. The value was practically unchanged, however, when the

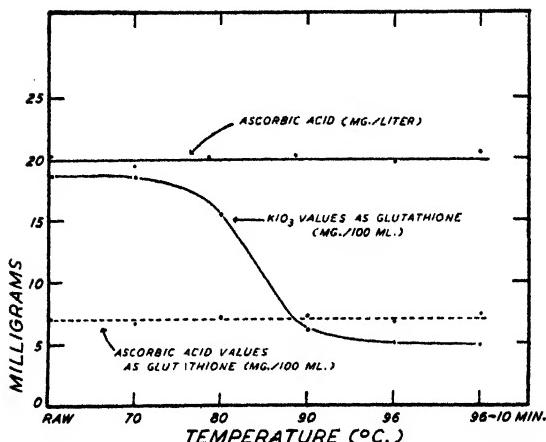


FIG. 1. The influence of heat on the  $\text{KIO}_3$  values and ascorbic acid of milk.

milk was heated to 70° C. The largest drop in the  $\text{KIO}_3$  values occurred between 80° C. and 90° C., and the higher temperature of 96° C., or 96° C. for ten minutes, caused only a relatively small further decrease. The graph shows also, that the ascorbic acid content as determined by 2,6-dichlorophenolindophenol remains substantially unaltered by the heat treatment.

The fact that the heat treatment had no definite effect upon the ascorbic acid indicates that the changes which occur in the  $\text{KIO}_3$  values are due to decomposition or rearrangement of the other reducing substances involved. Further, since temperatures above 90° C. brought only slight further decreases in the  $\text{KIO}_3$  values, the indications are that the bulk of the reducing material which is being acted upon has undergone change, and that the titer exhibited by this heated milk is due practically entirely to ascorbic acid alone. That this is true is illustrated in figure 1 by the line showing the ascorbic acid content expressed in its equivalent as mg. of glutathione per 100 ml. of milk. It may be observed that the ascorbic acid somewhat more than accounts for the  $\text{KIO}_3$  values of the milk heated to 90° C. or above.

#### DISCUSSION

The data assembled in this experiment show conclusively that milk contains reducing substances, other than ascorbic acid, which are measurable by potassium iodate. These reducing substances contribute varying proportions of the total reducing capacity of milk, ranging from 40 to 70 per cent of the total. In all cases encountered in this and subsequent studies, the total of the  $\text{KIO}_3$  values have always exceeded that which is contributed by the ascorbic acid alone. Conclusions should not be drawn at the present time as to what substance or substances are responsible for this reducing action of milk, but the proteins of the milk serum may be involved.

Sulphydryl linkages are not found in milk, consequently it may be logically assumed that neither cysteine, glutathione, nor thioneine contribute to the  $KIO_3$  values. If glutathione were involved, the treatment of the milk with copper would have affected the titration values of the non-ascorbic acid portion of the reducing materials. Although lactose is a reducing sugar, it is unlikely that it contributes to the reducing action, especially at the low pH at which the  $KIO_3$  determination is conducted.

It is interesting to note that the reducing power of the substance responsible for the  $KIO_3$  reducing ability of milk in excess to that contributed by the ascorbic acid is destroyed by heat at temperatures which cause the liberation of volatile sulfides from milk (3). This may be a coincidence. However, if proteins are involved, then consideration should be given the possibility that the substance contributing to the heat labile sulfides is also responsible for the bulk of the  $KIO_3$  titer.

#### CONCLUSION

Potassium iodate titration values of milk are greater than they should be if ascorbic acid alone were responsible.

The material in milk responsible for the iodate reducing power of milk in excess of that caused by the ascorbic acid is unaffected by sunlight or copper under the conditions of this experiment, but is slightly affected by treatment of the milk with iodoacetate.

$KIO_3$  values of milk are not changed by zinc dust reduction of a sulfo-salicylic acid filtrate.

The potassium iodate values of milk are lowered when milk is heated to 80° C. or above. This lowering is likely due to destruction or rearrangement of the non-ascorbic acid portion of the reducing materials.

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# THE DISAPPEARANCE OF ADDED GLUTATHIONE IN MILK\*

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Previously (1), the author observed that when 5 mg. of glutathione were added to milk, it was not detected by the nitroprusside test after a 15-minute incubation period. However, the same amount of glutathione added to milk which had been heated to 76° C. or above produced a positive nitroprusside reaction. This indicated the possibility that raw milk destroys glutathione, whereas milk which has been heated sufficiently high fails to exhibit this destructive property. Consequently, this study was conducted to determine quantitatively the disappearance of glutathione when added to raw and heated milk.

Oberst (2) found reduced glutathione to disappear when added to solutions containing egg albumin, casein and blood serum. He found both reduced and total glutathione content to decrease rapidly during the first 2-3 hours of incubation when added to whole blood. When glutathione was added to blood serum, the reduced glutathione concentration changed from 50.6 mg. per cent to 18.4 mg. per cent in 15 minutes, whereas the total glutathione content was reduced from 55.2 mg. per cent to 36.2 mg. per cent during the same period. Oberst suggests that the reduction of glutathione in protein solutions is due to chemical reaction, the glutathione combining with the protein so as to inactivate the sulfhydryl group.

## EXPERIMENTAL PROCEDURE

Milk used for this experiment was secured directly from disease-free cows at the college farm. Porcelain pails were used to collect the milk in order to eliminate metal contamination. The milk was held at 4° C. until used.

Glutathione (Pfanstiehls) was added to the milk at the rate of 50 mg. per cent and was thoroughly incorporated by stirring the milk frequently during the first 15 minutes of the incubation period. The glutathione determination, conducted essentially by the method of Woodward and Fry (3) as outlined in an earlier paper (4), was made on the milk after 15 minutes, 1 hour, 2 hours and 4 hours of incubation at 20° C.

Heating of the milk was conducted in a round bottom flask suspended in a boiling water bath. Samples of milk were withdrawn by means of a glass-tube siphon. Temperatures were accurate within  $\pm 0.5^\circ$  C.

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## RESULTS

*Addition of glutathione to raw milk:* Three samples of milk were secured from individual cows. Glutathione was added at the rate of 50 mg. per cent. The rate of disappearance of the reduced glutathione is illustrated by figure 1. The results are expressed as the actual glutathione present;

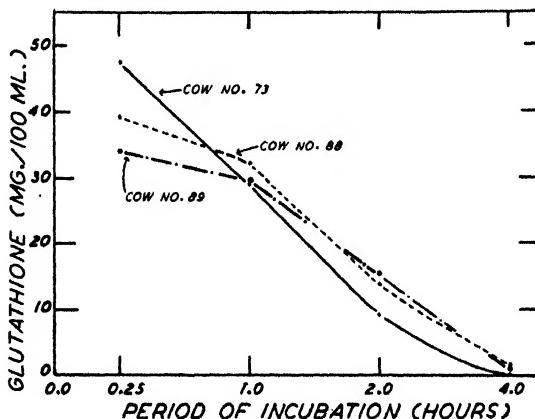


FIG. 1. The disappearance of reduced glutathione in raw milk (GSH added at rate of 50 mg. per cent).

consequently corrections are made in every case for the original  $KIO_3$  values of the milk.

Figure 1 shows the loss of glutathione in milk to proceed at a rapid rate. Following the 15-minute interval the glutathione values are considerably below the 50 mg. level. This is especially true of two of the samples. The reduced glutathione disappeared at approximately the same rate in all of the samples during the 4-hour incubation period. At the close of the 4-hour period the glutathione had decreased to values less than 2 mg. per cent. In fact, one sample showed complete loss of the glutathione at the close of the incubation period.

*Addition of glutathione to heated milk:* Glutathione was added at the rate of 50 mg. per cent to raw milk and to the same milk which had been heated momentarily to 70° C., 80° C., and 90° C. The results are shown in figure 2.

This figure illustrates the stabilization effect of the previous heat treatment of the milk on the glutathione. The raw milk shows rapid disappearance of the glutathione, similar to that shown in figure 1. Heating of the milk to 70° C. retarded, but did not prevent, the disappearance of the glutathione. However, when the milk had been subjected to temperatures of 80° C. or 90° C., it exhibited no destructive action on the glutathione. Actually, there was a slight increase in the glutathione values of these samples during

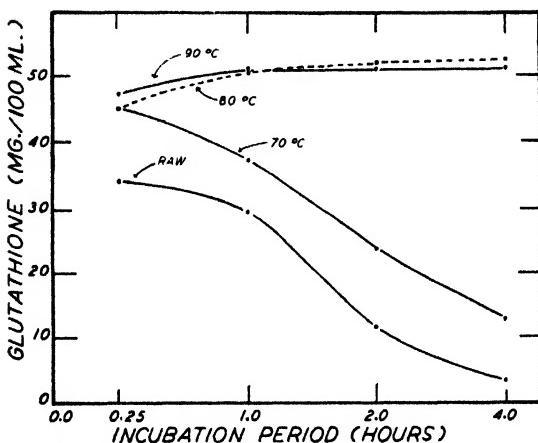


FIG. 2. The stabilization effect of heat on glutathione in milk (GSH added at rate of 50 mg. per cent).

the 4-hour period. This slight increase may be due to changes in the milk which have been brought about by the heat treatment.

*Changes in the reduced and total glutathione content in milk and milk serum:* The disappearance of reduced glutathione in milk raises a question; i.e., whether the glutathione is merely changed to the oxidized form, or whether it is entirely destroyed. Steps were taken to determine which of these processes occurs.

Glutathione was added at the rate of 50 mg. per cent both to whole raw milk and to rennet serum prepared from the same milk. The reduced and

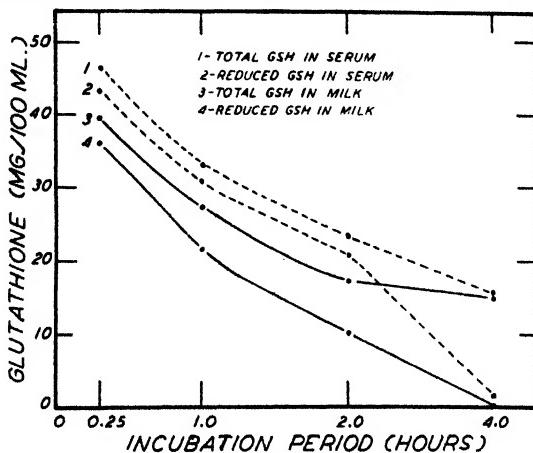


FIG. 3. The changes of reduced and total glutathione content in raw milk and in raw milk serum (GSH added at rate of 50 mg. per cent).

total glutathione content was determined after incubation periods as in the previous experiment. The total glutathione content was determined by reducing a portion of the sulfosalicylic acid filtrate with zinc dust according to the method of Woodward and Fry (3). Thus the difference between the reduced and total glutathione content represents the concentration of the oxidized form of glutathione. The results of this experiment are shown by figure 3.

The figure shows both the reduced and total glutathione values of the milk serum to be above those for the whole milk. The difference between the serum and milk values at the beginning of the incubation period is approximately 6-8 mg. of glutathione per 100 ml. This slightly higher value in the serum may be due partly to the dilution effect of the milk casein in the case of the whole milk or to a slight tendency of the casein to combine with the glutathione. However, the slight difference obtained between the two systems indicates that the casein *per se* plays no significant part in causing the disappearance of the glutathione.

Figure 3 shows further that the rate of disappearance of reduced glutathione is approximately as great in the serum as in the milk, although there was a slightly slower disappearance in the case of the serum.

The third important fact shown by the graph is that the major portion of the reduced glutathione which disappears in the milk is completely destroyed and is not merely changed to the oxidized form. The reduction of the acid filtrate by zinc dust at the various time intervals does increase the  $KIO_3$  titer, indicating that a portion of the glutathione is in the oxidized form. However, the difference between the total and reduced glutathione after 0.25 hour and 1 hour in the case of the milk, and after 0.25 hour, 1 hour, and 2 hours in the case of the serum is relatively small, amounting roughly to about 2-4 mg. of glutathione per 100 ml. A much wider difference between the total and reduced glutathione values was secured at the 4-hour period, the difference amounting to about 15 mg. The total glutathione content appears to be leveling off at about this value at the close of the incubation period.

#### DISCUSSION

The actual cause for the destruction of glutathione when it is added to raw milk can only be speculated upon. The fact that temperatures of 80-90° C. stabilize the glutathione may be taken to indicate that the destruction is enzymic in nature. However, one should not be too prone to accept this as being the proper explanation, since these temperatures are sufficiently high to cause the formation of volatile sulfides, thus producing a reducing system which may be capable of preventing destruction of the glutathione. The temperatures which were effective in preventing the destruction, however, are high enough to destroy the principal oxidizing enzymes of milk.

The proteins of milk may be involved in the disappearance of the glutathione, although the casein is apparently of little significance in this connection. If the albumin and globulin of milk are concerned with the destruction of the tripeptide, the stabilization effect of heat may be due to changes in these proteins which occur, and which may, in turn, be correlated with sulfide liberation.

The results herein reported show that when 50 mg. of glutathione are added to 100 ml. of milk or milk serum the glutathione is practically gone at the end of the 4-hour incubation period. These changes were observed by using the nitroprusside test as well as by the quantitative method, although only the quantitative results are reported. In other trials in which 100 mg. of glutathione were used, considerable glutathione remained at the close of 4 hours at 20° C. This would indicate that the destroying agent in the milk is incapable of causing as complete destruction of this high concentration of glutathione in the 4-hour interval as it does with concentrations of 50 mg. per cent.

#### CONCLUSIONS

Raw milk or rennet serum from the milk causes a rapid disappearance of added reduced glutathione.

The major portion of the glutathione which disappears is destroyed, especially during storage periods up to 2 hours, since only a relatively small quantity was found to be in the oxidized form. After 4 hours of storage, however, a greater proportion of the missing glutathione is found in the oxidized form.

Heating milk momentarily to 80 or 90° C. and then cooling, prior to adding the glutathione, resulted in complete stabilization of glutathione.

A lower temperature of 70° C. was less effective in preventing its disappearance.

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## PROTECTIVE INFLUENCE OF GLUTATHIONE ON COPPER-INDUCED OXIDATION OF ASCORBIC ACID IN MILK\*

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The ability of glutathione to be easily oxidized and reduced has aroused much interest as to its function in those biological tissues and fluids where it is normally present. During the past few years, many valuable contributions have resulted from research pertaining to the role of glutathione in biological systems. Of especial interest, however, insofar as this paper is concerned, are those findings which indicate a possible close relationship between glutathione and ascorbic acid.

Hopkins and Morgan (6) found glutathione to protect ascorbic acid from oxidation by copper. This protective action was ascribed as being due to the fact that the glutathione "forms a stable compound with the copper, preventing efficient contact between the metal and its substrate."

Barron, Barron, and Klemperer (1) also found glutathione to efficiently inhibit copper catalysis of ascorbic acid and expressed the belief that this inhibition may be due to the strong affinity of glutathione for copper to form a copper-glutathione complex. These workers found practically complete inhibitory action by glutathione as long as there existed more than one molecule of glutathione per atom of copper.

Differences of opinion exist as to the ability of glutathione to prevent enzymic oxidation of ascorbic acid. Hopkins and Morgan (6) observed that glutathione protected ascorbic acid from oxidation by the enzyme "hexoxidase," but that the glutathione was itself oxidized. This protective action persisted until the glutathione had substantially disappeared from the system. Stotz, Harrer, and King (9) attribute the catalytic oxidation of ascorbic acid in certain plant juices to copper present in combination with protein material, rather than to a specific "oxidase," and found that a mixture of copper and albumin assumed the characteristic properties of these suggested enzymes. However, in later work these workers found guinea pig liver brei brought about slow aerobic oxidation of ascorbic acid, which they attributed to the "indophenol oxidase-cytochrome system." The glutathione naturally present in the liver brei was unable to protect added ascorbic acid from oxidation. This finding may indicate that glutathione is specific for copper catalysis of ascorbic acid, a view held by Barron, *et al* (1).

No studies have been reported on the inter-relationship of glutathione and ascorbic acid in milk, possibly because glutathione has never been shown

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to be a normal constituent of this product. It appeared desirable, however, in conjunction with other studies (4), to determine if added glutathione would protect ascorbic acid against copper-induced oxidation. Results of this study may help to explain the stabilization effect of high temperature heat treatment of milk on ascorbic acid, such treatment resulting in the formation of reducing compounds (5, 7). In addition, knowledge of the glutathione-ascorbic acid relationship in milk may be applied also to other biological systems.

#### EXPERIMENTAL PROCEDURE

Milk used for this experiment was obtained from individual cows of the Michigan State College herd. The milk was collected directly in procelain containers, cooled and placed in glass bottles. In order to study the protective action of glutathione, it was necessary to prevent the disappearance of the glutathione which occurs naturally in raw milk (4). This was accomplished by heating the milk in a glass flask to 80° C., a temperature which was previously found to be sufficiently high to inhibit this disappearance (4). Immediately after the heat treatment, the milk was cooled and divided into four lots. The lots were treated as follows:

Lot 1—Control.

Lot 2—1.5 p.p.m. of copper as copper sulfate.

Lot 3—1.5 p.p.m. of copper plus either 25 or 15 mg. per cent of glutathione (Pfanstiehls).

Lot 4—Either 25 or 15 mg. per cent of glutathione.

The samples were stored at 4° C. and examined for ascorbic acid and glutathione after 15 min., 1 hr., 2 hrs., 4 hrs., 6 hrs., 24 hrs., and 48 hrs. The ascorbic acid determinations were made by titration with 2, 6-dichlorophenolindophenol according to the method of Sharp (8). The glutathione determinations were conducted by the macro  $\text{KIO}_3$  titration procedure used previously (3) and adapted from the micro method of Woodward and Fry (11). Corrections were made for the potassium iodate titer of the control sample in order to arrive at the actual glutathione content of the samples in Lots 3 and 4.

#### RESULTS

The first portion of the experiment consisted of studying the influence of 25 mg. per cent of glutathione on stabilizing the ascorbic acid against copper catalysis. As stated in the procedure, the milk used in the trials had previously been heated to 80° C. to inhibit the disappearance of the added glutathione which occurs in normal raw milk. It is recognized that such heat treatment will tend to stabilize the ascorbic acid (2, 7). The results from individual trials were practically identical. Consequently, averages were secured and are graphically shown in figure 1.

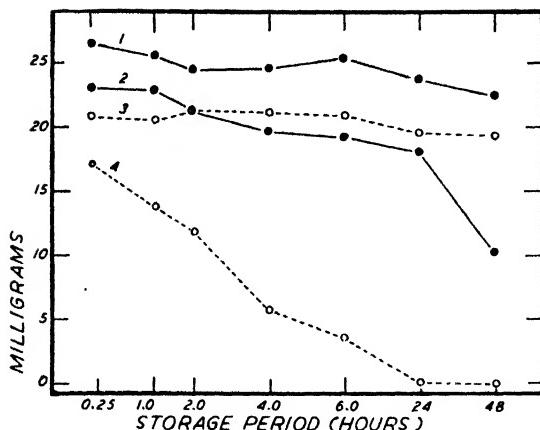


FIG. 1. The ability of 25 mg. per cent of glutathione to prevent copper-induced oxidation of ascorbic acid. (Ascorbic acid as mg. per liter, glutathione as mg. per 100 ml.).

Curve 1—Glutathione in sample containing no copper.

Curve 2—Glutathione in sample containing 1.5 p.p.m. of copper.

Curve 3—Ascorbic acid in sample containing glutathione and 1.5 p.p.m. of copper.

Curve 4—Ascorbic acid in sample containing 1.5 p.p.m. of copper but no glutathione.

This figure shows glutathione, in the concentration used, to greatly protect the ascorbic acid from copper catalysis throughout the 48-hour storage period. The ascorbic acid values remained substantially unchanged in the milk containing both copper and glutathione, whereas it had practically disappeared within six hours in the lot which contained copper but no glutathione. Although the ascorbic acid values for the control lot are not shown, they changed but slightly during the storage period, usually decreasing only 2 to 3 mg. per liter.

Figure 1 also shows the changes in the actual glutathione content in the lot which contained added copper and in that to which no copper was added. The GSH content of the milk which contained no added copper remained practically uniform throughout the period, the slight decrease which did occur amounting to approximately 4 mg. However, in the presence of copper the GSII slowly but definitely disappeared. The GSH value had dropped from approximately 23 mg. per cent to 18 mg. per cent during the first 24 hours, and had decreased still further to approximately 10 mg. per cent by the close of the 48-hour period. Considering that 25 mg. per cent of glutathione had been added to the milk, the results indicate that approximately 60 per cent of the reduced glutathione had disappeared.

Somewhat similar results were secured when 15 mg. per cent of glutathione were added to the milk. This is shown by figure 2. In these trials, however, the ascorbic acid was not as completely protected as in those in

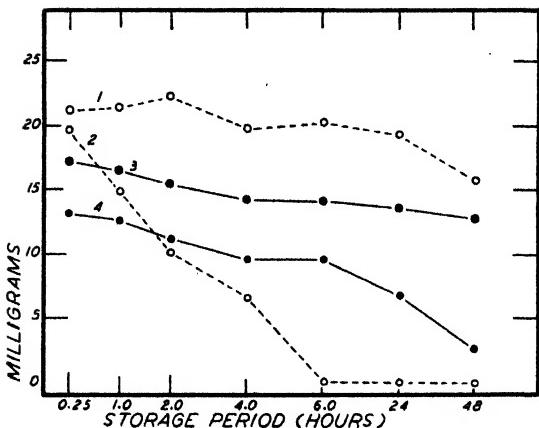


FIG. 2. The ability of 15 mg. per cent of glutathione to prevent copper-induced oxidation of ascorbic acid. (Ascorbic acid as mg. per liter, glutathione as mg. per 100 ml.).

- Curve 1—Ascorbic acid in sample containing glutathione and 1.5 p.p.m. of copper.
- Curve 2—Ascorbic acid in sample containing 1.5 p.p.m. of copper but no glutathione.
- Curve 3—Glutathione in sample containing no copper.
- Curve 4—Glutathione in sample containing 1.5 p.p.m. of copper.

which 25 mg. per cent of GSH were used. The ascorbic acid content of the milk containing 15 mg. per cent GSH plus copper showed slight decreases after 24 and 48 hours; the total decrease amounting to approximately 5 mg. In contrast to this, the milk containing copper but no glutathione had lost all of its ascorbic acid after 6 hours.

Figure 2 again shows disappearance of reduced glutathione in the milk containing added copper, the GSH content dropping from 23 mg. per cent after 15 minutes to approximately 3 mg. after 48 hours. Therefore, the total loss in GSH amounts to approximately 12 mg. per cent, or 80 per cent of the total which was added. The glutathione in the milk which contained no added copper showed a slight loss of approximately 2 mg. per cent.

In connection with the glutathione content of the milk to which no copper was added, it may be observed in both figures that the values after 15 minutes are slightly higher than would be expected on the basis of the 25 and 15 mg. per cent which were added. This slightly higher value has been observed many times, and likely results from changes which have occurred in the milk due to the heat treatment to which it was subjected.

#### DISCUSSION

The results secured in this study show that when glutathione is added to milk, previously heated to 80° C., it exhibits marked protective action against copper catalysis of ascorbic acid oxidation. The concentrations of gluta-

thione used in this study, *i.e.* 15 and 25 mg. per cent, were sufficient to almost entirely prevent oxidation of the ascorbic acid for the 48-hour period as induced by 1.5 p.p.m. of copper. The inhibiting power of 25 mg. per cent glutathione was sufficient to inhibit all oxidation of the ascorbic acid, whereas the 15 mg. per cent appeared to be somewhat less effective.

Although the ascorbic acid was not oxidized in the presence of the glutathione and copper, the glutathione itself was affected. This indicates that the glutathione is removed from the system by copper either by oxidation or combination. In the trials in which 15 mg. per cent of glutathione were added, the slight loss of ascorbic acid may have resulted due to the fact that the major portion of the glutathione had been destroyed, thus allowing the copper to oxidize the ascorbic acid. It is interesting to note that the proportion of glutathione to copper in these trials far exceeds the proportions of one molecule GSH: one atom copper which Barron, *et al* (1) found sufficient to prevent ascorbic acid oxidation.

#### CONCLUSIONS

The addition of 25 mg. per cent of glutathione to milk previously heated to 80° C. inhibited copper oxidation of ascorbic acid over a 48-hour period. Fifteen mg. per cent of glutathione was only slightly less efficient in preventing the catalytic action of copper.

Although the glutathione prevented copper catalysis of the oxidation of ascorbic acid, it slowly disappeared from the system.

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# CHANGES OBSERVED IN MILK "SHAM FED" TO DAIRY CALVES<sup>1</sup>

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The specific digestive rôles played by saliva and esophageal secretions of ruminants are somewhat obscure. Since the saliva of the bovine presumably contains insignificant amounts of enzymes, the primary functions generally ascribed to the mixed secretions in the mouth are restricted to those of a physical nature. The importance of these secretions is readily recognized when the diet consists of solid foods but is probably under evaluated when the diet is liquid. Yet milk is listed among the food substances that produce the richest flow of saliva (1).

As is commonly observed, the young calf secretes copious amounts of saliva, particularly when nursing. It is reasonable to assume that the secretions of the oral cavity and esophageal passage serve an important rôle in the assimilation and utilization of milk by the animal. Espe (5) observed that ingested milk collected from an esophageal fistula increased markedly in viscosity, the maximum being reached about seven minutes after collecting and finally disappearing by the end of seven hours. He further suggested that this initial increase in viscosity aids in retaining the milk in the abomasum until rennet and/or acid coagulation is complete.

The foregoing observations and suggestions prompted an investigation designed to discover some of the changes produced in consumed milk prior to its entrance into the stomach of the dairy calf.

## EXPERIMENTAL METHODS

*Experimental Subjects.* Four fistulated calves, described in table 1, were employed in this investigation. The three rumen fistulae were established according to procedures already described (12) except for one modification; that is, instead of waiting for the rumen wall to heal to the skin before excising the exposed area of the rumen wall, the opening was made immediately after the wall was sutured to the skin.

The gun-barrel esophagostomy operative technique used was similar to that described by Espe (5). The calf was completely anaesthetized by intravenous injections of a chloral hydrate solution. Subsequently, the incision area, a region of the neck between 10 and 20 centimeters posterior to the angle of the jaw, directly over the jugular groove and slightly below the jugular vein was shaved, cleansed and disinfected. A five-centimeter longitudinal incision paralleling the esophagus was made through the

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TABLE 1  
*Experimental subjects and systems of milk feeding*

Herd No.	Breed	Age	Type of fistula	Systems of feeding milk
C-55	Holstein	147	Ruminal	Alternating*: Open pail and nipple
A-117	Jersey	46	Ruminal	Alternating: Open pail and nipple
A-119	Jersey	43	Ruminal	Alternating: Open pail and nipple
42	Guernsey	22	Esophageal	Nipple only

\* Each system was used one day, two feeds, before changing.

skin and underlying fascia. The muscles were separated, and the esophagus was freed from the surrounding tissue by blunt dissection, taking precaution to avoid breaking any of the blood vessels. The esophagus was severed; the two free ends were drawn to the surface and sutured around the circumference to the skin at the respective extremities of the incision, the terminations of which were rounded to fit the curvature of the esophagus. The edges of the skin incision between the ends of the esophagus, a distance of about two centimeters, were also sutured. The wound was dressed daily until healing resulted, at which time the stitches were removed.

A rubber tube, inserted into the exposed ends of the esophagus and held in place by a string attached to the wall of the tube and passing around the neck of the calf, served as a conduit for normal milk feeding.

**Experimental Feeds.** Pasteurized whole milk, superheated pasteurized whole milk, unpasteurized separated milk and reconstituted separated milk were fed in the various experiments. Pasteurized milk was heated at 63°–64° C. for 30 minutes, and the superheated milk was heated an additional five minutes at 80° C. in order to inactivate any lipase that might have survived pasteurization. The superheating and the cooling were done immediately before feeding. The reconstituted separated milk consisted of one pound of spray-dried milk in nine pounds of water.

All milk fed, except the reconstituted, was from a composite of the entire daily production of a herd of approximately 100 cows in various stages of lactation. The average test of the whole milk was 4.3 per cent fat and that of separated was 0.05 per cent fat. Whenever it was necessary to store the milk, it was held at a temperature of 35° F. In no case was the milk retained for experimental feeding longer than 10 hours after pasteurization or separation.

Immediately preceding feeding, a sample of the unconsumed milk, control, was taken for comparing with the consumed, "sham-fed." These samples, control and "sham-fed," were subjected to the same tests under standard conditions.

*Procedures for Sham Feeding Milk.* The calves having rumen fistulae were fed alternately from open pails on the floor and nipple calf feeders\* in an elevated position; whereas the esophagostomized calf was fed by the latter system only. The milk was fed at a temperature of 100° F. twice daily, the quantity at each feed amounting to approximately seven per cent of the body weight. The time required to ingest a given weight of milk by either system was determined by means of a stop watch.



FIG. 1. Method of "sham feeding" milk via cardial end of esophagus (rumin fistulated calf).

As the milk was consumed, it was withdrawn from either the cardial end (figure 1) or the medial section (figure 2) of the esophagus. In the first procedure, a glass tube having a rubber conduit attached was inserted approximately two inches into the posterior extremity of the esophagus, the entrance being made progressively *via* fistula, ruminal cavity and cardia. In order to prevent expulsion of the tube during deglutition, the conduit was held in place by hand. Thus a continuous passage was formed permitting the milk to flow from the mouth through the esophagus and conduit into an exterior receptacle.

As a means of ascertaining whether or not the point of the esophageal outlet was a factor to be considered, the milk was withdrawn by the second method (figure 2). During the collection period, the conduit was in the posterior end of the pharyngeal section of the esophagus.

\* Coyner feeder pail, sold by the Armour & Company, U. S. Yards, Chicago, Ill.



FIG. 2. Method of "sham feeding" milk *via* medial section of esophagus (esophago-stomized calf).

In all collections from the conduit the first two or three swallows of milk, which contained much accumulated saliva and some residues of solid feeds, were discarded. The remainder of the material was received as shown in figures 1 and 2 and subsequently examined.

*Technique employed in examination of milk.* Representative samples of control and consumed milks were subjected within 10 minutes after collection to the following examinations in the order listed: (a) hydrogen-ion concentration, (b) rennet coagulation, (c) curd tension, (d) lipolytic activity and (e) gravity creaming. In several cases, samples of milk were retained at a constant temperature of 38° C. for two hours, after which several of the tests were repeated.

*A. Hydrogen-ion concentration.* This was measured with a Beckman, glass electrode pH meter. Since the pH of the consumed whole milk changed rapidly, this determination was made within two minutes after collection.

*B. Rennet coagulation.* Except for the modification of the temperature at which the milk was held, the procedures employed in this determination were as outlined by Sommer and Matsen (11). Since the body temperature of cattle is approximately 38° C., this degree of heat was considered preferable, from a physiological point, to the prescribed 30° C. for tempering the milk.

*C. Curd tension.* The methods used for measuring curd strength were the same as recommended by the committee on Methods of Determining Curd

Tension of Milk (2) except that the milk was adjusted to a temperature of 38° C. instead of 35° C. The measuring apparatus used was the Submarine Signal Curd Tension Meter.

*D. Lipolytic activity.* The activity of lipolytic enzymes in the various samples of milk was estimated according to the general procedure of Nair (9). Five mls. of milk were added to 100 mls. of a sterilized sucrose-cream substrate. This was incubated at 37° C. for 21 days, during which period determinations of the increases in titratable acidity were made at various intervals. Since the rate of increase generally was retarded after six to eight days, the lipolytic data recorded in the tables represent the measurements on the seventh day. The lipolytic activity was expressed in terms of mls. of 0.1N · NaOH required to bring 10 gms. of the substrate admixture to the neutral point as indicated by phenolphthalein.

*E. Gravity creaming.* The cream volume, or layer, was used as an index of the relative stability of the emulsions of various milk samples. The extent of the fat rising was estimated on samples placed into 100 ml. graduated cylinders and held at room temperature for 24 hours, after which the depth of the so-called cream layer was measured.

#### EXPERIMENTAL RESULTS

The experimental observations involved several variables: pre-feeding treatment of milk, individual calves, and systems of "sham feeding," which were varied by the method of administration and by the point of collection from the esophagus. The data as presented in tables 2 to 5, inclusive, suggest that several of these variables were insignificant factors; whereas others were of primary importance.

*Relation of feeding system to the changes produced in the milk.* When similar systems of feeding were employed, the trend of changes noted in whole milk was essentially the same when collections were made from the cardial end of the esophagus (table 2) as when made from the medial region (table 3). These results indicated that if the secretions from any one section of the esophagus contribute to the alterations of the milk, the pharyngeal end is the primary region involved.

The consumed milk, irrespective of method of feeding, exhibited several properties markedly different from those of the control. A comparison of the magnitude of the alterations resulting from the two systems of feeding indicated that in whole milk the changes of all observed properties were more exaggerated when fed by the nipple system than from open pail (tables 2 and 4), but in separated milk there was no marked difference except in lipolytic activity (table 5).

*Individual calves as a factor in the alterations of milk.* The data in tables 2, 4 and 5 reveal considerable variation in the magnitude of the changes in milk "sham fed" to different individuals. The modification of

TABLE 2  
*Comparative changes observed in "sham fed," pasteurized whole milk subjected to various tests immediately after collection from the cardinal end of the esophagus*

Method of feeding	Herd No. of calf	Observations	Rate of cons.	Curd tension		pH	Lipolytic activity	Cream volume per cent
				Rennet coag. time min.	Surface gms.			
Control	..	10	lbs./min. -	10.53	40	6.39	mls. NaOH 0.53	8.8
C-55	5	16.6	"	6.35	47	6.32	1.97	32.7
A-117	5	3.8	"	3.04	45	6.01	4.33	69.8
A-119	5	2.1	"	4.36	44	6.22	2.35	45.3
Average	"	7.5	"	4.58	45	6.18	2.88	49.3
C-55	5	2.3	"	3.26	49	6.00	3.50	69.8
A-117	5	1.6	"	1.95	50	5.93	4.46	98.0
A-119	5	0.9	"	1.93	47	5.81	4.95	89.5
Average	"	1.6	"	2.38	49	5.91	4.30	85.8

TABLE 3  
*Comparative changes observed in various samples of milk "sham fed," via esophageal fistula and tested immediately after collection*

Kind of milk	Treatment of milk	No. of observers	Rennet coag. time <sup>a</sup>	Curd tension		pH	Lipolytic activity	mls. NaOH per cent	Cream volume
				min.	Surface Body gms.				
Pasteurized whole	Control	2	11.20	41	35	6.30	—	0.90	9.0
	Consumed	2	3.63	43	43	5.70	—	5.25	99.0
Reconstituted skim	Control	1	17.51	7	7	6.18	0.60	—	—
	Consumed	1	16.90	11	11	6.20	5.95	—	—

TABLE 4  
*Comparative changes observed in "sham fed," superheated whole milk subjected to various tests immediately after collection from the cardial end of the esophagus*

Method of feeding	No. of expt. subj.	No. of observ.	Milk fed	Rate of cons.	Rennet coag. time	Curd tension		pH	Lipolytic activity
						Surface	Body		
Controls		2	A B	-	lbs./min. min.	20.98 19.96	19 25	gms. 19	mls. NaOH 6.37 6.48
C-55	1	1	B		16.5	19.39	26	26	6.44 2.50
A-117	2	A	4.4		15.06	23	22	6.18	5.47
A-119	1	B	3.6		19.11	28	28	6.42	2.65
Average difference from controls*					- 3.31	+3	+3	-0.11	+3.45
C-55	2	A	3.3		17.87	27	25	6.20	4.73
A-117	1	B	2.6		7.58	25	25	6.49	4.85
A-119	2	A	1.4		15.51	25	25	6.17	4.67
Average difference from controls*					- 5.91	+6	+5	-0.18	+4.13

\* Since controls varied considerably, the average for "sham-fed" milks is expressed in terms of difference from their respective controls.

TABLE 5  
*Comparative changes observed in "sham fed," unpasteurized separated milk subjected to various treatments immediately after collection from the cardinal end of the cisternum*

Method of feeding	Herd No. of calf	Observations	Rate of cons.	Rennet coag. time	Curd tension		pH	Lipolytic activity	m.s. NaOH
					min.	Surface Body			
Control		4	lbs./min.	10.89	64	61	6.48		1.29
C-55	2	22.7		10.03	67	58	6.38		2.97
A-117	2	4.3		9.80	57	48	6.55		5.67
A-119	2	4.0		9.76	56	49	6.43		3.03
Average		10.3		9.86	60	52	6.45		3.90
C-55	2	3.0		10.61	65	56	6.55		4.55
A-117	2	0.3		8.98	58	43	6.37		5.55
A-119	2	1.7		10.16	54	46	6.52		5.65
Average		2.3		9.92	59	48	6.48		5.25

the milk was less pronounced in the case of C-55, an older calf, than in the case of either A-117 or A-119. Though these last two were about the same age, there was a marked difference in vigor. E-219 was somewhat unthrifty and apparently secreted less saliva. Closely associated with the degree of change in the milk consumed by different calves were the rates of consumption. Whether or not this relationship is an *a priori* condition remains to be determined. Since the system of feeding each calf was alternated from day to day, the variations attributable to this factor are compensatory and consequently do not play an important rôle in the average.

*Effect of pre-feeding treatment of milk on the changes resulting from sham feeding.* When pasteurized whole milk was "sham fed," the properties of the milk were markedly altered as indicated in tables 2 and 3. These changes involved a pronounced reduction in rennet coagulation time, a slight increase in curd tension, a striking decrease in pH, an increase in cream volume and an augmentation of lipase activity. The trend of the changes for "sham-fed" superheated whole milk (table 4) were essentially the same as for the pasteurized, but the extent of the modification was somewhat less, except in lipolytic activity. In the case of unpasteurized separated milk (table 5) there was a slight reduction in rennet coagulation time, a tendency to diminish the strength of the body of the curd, a rise in lipolytic activity but no significant change in pH. "Sham feeding" reconstituted skim milk (table 3) produced few definite changes other than an increase in lipolytic activity. Since the number of determinations was exceedingly limited, no specific significance can be ascribed to the apparent increase in curd tension, which in the control was relatively low.

In accordance with reports of other investigators, a comparison of the various samples indicates that superheating greatly increased the rennet coagulation time (10, p. 230) and reduced curd tension (2); whereas removal of fat apparently strengthened the curd tension, surface and body, but did not affect perceptibly the rate of rennet coagulation.

*Changes in the properties of various samples of milk retained two hours.* In order to determine the comparative changes in the milk over a period of time, the various control and "sham-fed" samples were retained at 38° C. for an arbitrary period of two hours. After this time, the tests were repeated. A comparison of the changes observed immediately after "sham feeding" with those made two hours later is presented in table 6. In the case of the pasteurized whole milk little change was noted in the control, but in the "sham-fed" there was a continued reduction in the rennet coagulation time, a diminution in the curd tension, particularly in the case of the nipple system of feeding, and a further lowering of the pH. Except for a slight decline in the curd tension of the control sample, the changes in the superheated milk were essentially the same as for the pasteurized. In contrast with whole milks, the only conspicuous modification of the control separated

TABLE 6

*Relative changes observed in various samples of control and "sham fed" milks retained at 38° C. for a period of two hours*

Kind of milk	System of feeding	Time of observ.	No. of observ.	Rennet coagulation time	CURD TENSION		pH
					Surface	Body	
Pasteurized whole milk	Control	Immed.	5	10.52	40	39	6.35
		2 hrs.	5	11.18	38	37	6.51
	Open pail	Immed.	7	4.31	48	41	6.10
		2 hrs.	7	1.74	42	41	5.82
	Nipple pail	Immed.	8	2.35	49	44	5.90
		2 hrs.	8	1.24	31	31	5.56
Superheated whole milk	Control	Immed.	2	18.92	24	24	6.42
		2 hrs.	2	20.99	18	18	6.53
	Open pail	Immed.	3	17.38	27	27	6.34
		2 hrs.	3	9.94	21	21	6.17
	Nipple pail	Immed.	3	12.11	31	30	6.30
		2 hrs.	3	1.13	18	18	5.59
Unpasteurized skimmilk	Control	Immed.	2	11.23	65	63	6.54
		2 hrs.	2	10.51	57	53	6.53
	Open pail	Immed.	3	10.27	59	53	6.45
		2 hrs.	3	8.40	69	54	6.44
	Nipple pail	Immed.	3	10.36	59	48	6.55
		2 hrs.	3	8.19	69	53	6.48

milk was a decreased curd tension; whereas in the "sham-fed" sample there was a slight rise in curd strength and an acceleration in rate of rennet coagulation but no change in pH.

From general appearance the viscosity of all milks was augmented by "sham feeding." This reaction seemed to be more evident in whole milks, particularly the pasteurized, than in the separated. The viscosity of "sham-fed" milk increased rapidly, reaching the maximum within ten minutes, but abated slowly as evidenced by its persistence at the end of the two hours holding period.

#### DISCUSSION

In view of reported information relative to the properties of bovine saliva, it was logically assumed that its combination with whole milk by "sham feeding" would result in a mixture having a pH somewhere between the values for the two fluids, pH 8.1 for saliva and 6.6 for milk. Preliminary observations revealing a reduction of pH below that of the control milk were rather baffling; but subsequent experiments helped to clarify these paradoxical results. A pronounced rancidity accompanying the increased hydrogen-ion concentration suggested that lipolysis was involved, which indication was substantiated in subsequent tests. A comparison of the hydrogen-ion

concentration of "sham-fed" whole milk with that of "sham-fed" separated milk indicated that fat was a constituent essential for the change in reaction. Thus the decreased pH of the "sham-fed" whole milk becomes explainable on the basis of the liberation of fatty acids from the milk fat by lipolysis.

These results focused attention on the problem of ascertaining the origin of the lipolytic agents. Since saliva is generally considered to be devoid of lipase, attention was directed toward other sources. Inasmuch as milk frequently contains lipase (10, p. 30), the possibility that the increased lipolysis in "sham-fed" milk might have been due to the activation of enzymes present in the control milk was considered first. This conjecture was invalidated by the discovery that "sham-fed" milk previously superheated to insure inactivation of lipase manifested as much lipolytic activity as the "sham-fed" pasteurized milk.

Thus it appears that the lipolytic agents are in the fluids that are mixed with the milk in the process of consumption. Their origin evidently is either in micro-organisms present or in various secretory glands. Preliminary bacterial investigations of control and "sham-fed" milks showed a marked increase in total numbers of bacteria in the latter. This increase included some lipolytic organisms, which were hardly adequate to account for the pronounced lipolysis of the "sham-fed" whole milk. This suggestive evidence merits further investigation.

As to the glandular origin, the possibility that the lipolytic substances are extracellular enzymes cannot be excluded. Koebner (6) detected lipase in the saliva of sheep and oxen. Koldayev and Pikul (7) found that saliva from the parotid and submaxillary glands of dogs has definite lipolytic action. These reported observations in conjunction with the foregoing results are provisional evidence for postulating that the lipolytic activity detected in the "sham-fed" milks may be due to secreted enzymes. In addition to the probable involvement of the salivary glands, the glands in the pharyngeal end of the esophagus (4, p. 239), the function of which has not been established, are possibly also implicated.

The extent to which the various modifications of the "sham-fed" milks are attributable directly to lipolysis and the accompanying change in pH is not definite. The complex physical and chemical nature of the saliva and associated fluids no doubt influences the character of the milk in many ways. From a physical standpoint dilution is one of the direct effects.

As reflected in curd strength measurements, simple dilutions decrease the tension (2). The slight initial decrease of this value in the body of "sham-fed" separated milk was probably a manifestation of the dilution phenomenon, which in whole milk was evidently counteracted by increased acidity. This reaction according to Doan (2) toughens the curd as the pH drops from the normal (6.4 to 6.7) to approximately 5.9 but decreases curd tension from this point down to the isoelectric point (pH 4.7). This phe-

nomenon is best exemplified by the changes observed in whole milk "sham fed" from a nipple pail (table 6).

Before "sham feeding," the pH was 6.35 and curd tension for surface and body, respectively, was 40 grams and 39 grams. Immediately after feeding, the respective values were 5.90, 49 and 44, but two hours later the corresponding readings were 5.56, 31 and 31. Throughout, the changes in curd tension are small but relatively consistent, which contributes to their possible significance. Though lipolysis and dilution evidently are important factors affecting the curd tension of "sham-fed" milks, the changes probably should not be attributed to these two factors alone.

The retarding effect of dilution on rate of rennet coagulation was counteracted, in part, by increased acidity. As the pH decreased, there was an acceleration in the rate of coagulation, which observation is in accord with the results of other investigators (10, p. 232). That the increased acidity was not the only accelerating factor is suggested by the slight reduction in the coagulation time of "sham-fed" separated milk, the pH of which was not changed perceptibly.

Another characteristic of the "sham-fed" milks was their comparative stability as manifested by the slight fat rising in whole milk and by the prolonged time necessary for wheyng to start in both whole and separated milks. These properties probably are associated with the increased viscosity, a phenomenon also observed by Espe (5). Perhaps one of the primary constituents contributing to the viscosity and tending to maintain a uniform distribution of fat in the milk is the mucin of the saliva.

The quantity of saliva secreted, the extent to which it is mixed with the milk and probably the manner of mixing are factors affecting the degree of modification of "sham-fed" milks, particularly whole milk. The more copious the salivary flow and the more the secretions are mixed with the milk, the greater are the alterations. The first several swallows of milk come in contact with more saliva and other secretions than the remainder, due primarily to the pre-feeding salivary flow, a response to psychic stimuli. The constancy of the salivary flow after milk feeding is initiated remains an unsolved problem.

The amount of salivary secretion apparently varies with the age, with the manner of milk consumption and with the vigor of the individual calf. As the calves grow older, the rate of milk consumption accelerates without an apparent commensurate increase in salivary flow, thus resulting in less saliva per unit of milk ingested. The nipple system of feeding in contrast with the open pail method stimulates salivation and retards the rate of consumption, resulting not only in more saliva per unit of milk but also in more mixing. In addition it is possible that the agitation to which the milk is subjected during nursing promotes lipolysis (8). Suppression of saliva secretion apparently is associated with an unthrifty state of the individual, but *a priori* relationships have not been established.

According to Dukes (4, p. 234) the character of the food affects not only quantity but also quality of the saliva. It is possible that the milk excites secretions from glands that are not generally stimulated by other foods. Furthermore, the qualitative response of the salivary glands to any one dietary constituent perhaps is governed to some extent by the age of the animal. Therefore, if the lipolytic activity in "sham-fed" milks is due to a secretory product, it is possible that this reaction is restricted to young calves consuming milk.

It is difficult to assess the true significance of the foregoing *in vitro* observations of "sham-fed" milks in terms of specific *in vivo* reactions. However, it appears that digestion of milk fat is initiated prior to its passage into the stomach of the calf. The accompanying changes, including increased viscosity, probably aid in accelerating rennet coagulation in the abomasum, which reaction is in accord with Espe's theory (5). That is, quick and thorough coagulation is desirable to prevent flooding of the small intestine, which organ is very sensitive. The slightly increased curd tension observed does not necessarily adversely affect digestibility of the coagulum (3) as previously suspected. Seemingly, then, the initial ingestive changes in milk, whole milk particularly, facilitate its subsequent digestion.

The nipple feeding in comparison with the open pail system augments the degree to which "sham-fed" milk is modified. Nursing, in addition to aiding in the direct passage of milk to the abomasum (12) evidently promotes initial changes in the character of milk expediting subsequent digestion and utilization. The advantages of the nipple system in pre-gastric changes of milks apparently are not as striking in separated milk as in whole. However, this must not be considered as an imputation against the practice of feeding skimmilk by the nippé method. In the final analysis the foregoing fundamental observations increase credence in the value of the nursing system for feeding milk to young calves.

#### SUMMARY

1. Pasteurized and superheated whole milks and separated milk were "sham fed" either from open pails or through nipples to fistulated calves.

2. A comparison of the properties of various samples of "sham-fed" milks with properties of their respective controls revealed in the case of pasteurized whole milk increases in rate of rennet coagulation, in tension of the body of the curd, in hydrogen-ion concentration, in lipolytic activity and in cream volume. The corresponding changes in superheated whole milk were in the same direction but of less magnitude. Separated milk manifested a slight increase in the rate of coagulation, an insignificant decrease in curd tension, no pronounced change in hydrogen-ion concentration but a marked increase in lipolytic activity.

3. Further alterations were evident after retention of the samples at 38° C. for two hours. Except for a slight increase in pH there were no marked changes in the control sample of pasteurized whole milk, but in the "sham-fed" samples all initial changes, except for a reduction in curd tension, were further amplified. The trends of all samples of superheated whole milk were in the same direction as in pasteurized whole milk. In the separated milk the only notable change in the control sample was a decrease in curd tension, whereas in the "sham-fed" sample there was a slight increase in rate of coagulation and in curd tension.

4. Several of the pronounced changes in the properties of the whole milk as contrasted to those of the separated milk were attributed in part to lipolysis, a reaction probably resulting from enzymes present in saliva and/or other secretions of the mouth and esophagus.

5. Ingestion of milk through a nipple as compared with consumption from an open pail exaggerated the changes in whole milks but produced little difference, except in increased lipolytic activity, in separated milk.

6. The pre-gastric alterations of milk apparently are of such a nature as to expedite subsequent digestion and assimilation.

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## THE EFFECT OF PHOSPHORIC ACID SILAGE ON THE ACID-BASE BALANCE IN DAIRY COWS

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The use of mineral acids for the preservation of ensiled crops has raised the question of their physiological effects, particularly upon acid-base balance. There are several reports in the literature, dealing mostly with A.I.V. silage which contains hydrochloric acid or a mixture of hydrochloric and sulfuric acids. Hayden and co-workers (4) fed A.I.V. silage to dairy cattle as the only roughage for five months. A nearly complete disappearance of bicarbonates in the urine resulted while ammonia excretion increased as much as 40-fold. The CO<sub>2</sub> combining capacity of the blood plasma was slightly reduced. These effects were not cumulative and did not reach a dangerous point. Peterson and coworkers (8) and Bohstedt and coworkers (1) report similar changes in blood and urine values when feeding A.I.V. silage as the only roughage. The silage was supplemented with four ounces of limestone daily in one experiment and, in the second, with one ounce of a mixture of 10 parts of CaCO<sub>3</sub> to 3 parts of Na<sub>2</sub>CO<sub>3</sub> per fifteen pounds of silage. Changes occurring in the acid-base balance were considered to be of little significance, or at least not dangerous to the health of the animals.

Grasemann (3) likewise reports urinary acidosis and a reduced alkaline reserve of the blood plasma when feeding A.I.V. silage to dairy cows. He recommends supplementing the silage either with hay high in mineral elements or with CaCO<sub>3</sub> or NaHCO<sub>3</sub>. He remarks that CaCO<sub>3</sub> does not always produce the desired results, and that the addition of basic salts is justified even when feeding hay as the latter does not always contain a sufficient basic surplus. Bronwer and Dijkstra (2) report the same findings when feeding unneutralized A.I.V. silage. The extent of the physiological changes was much diminished when a neutralizing agent was added to the silage. Virtanen (12) fed several cows during a winter period with rations containing large amounts of silage preserved with hydrochloric acid. No disturbances were detected other than a change of the urine pH from the alkaline to the acid side and a great decrease in the amount of combined CO<sub>2</sub> excreted. When soda or a mixture of soda and limestone was included in the ration, the reaction of the urine remained close to normal.

Mollgaard and Thorbek (5) carried out energy metabolism studies on seven cows fed A.I.V. silage. They report that the acidosis developed by the addition of mineral acids to silage resulted in a negative calcium balance and increased enormously the heat production. This increased heat

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production was sufficient to decrease significantly the nutritive value of the fodder. The further statement is made that neutralization with basic salts restored a positive calcium balance and decreased the heat production, but that the digestibility of the ration was significantly depressed, especially when sodium bicarbonate was used. Supplementing the ration with beet roots, however, had a very beneficial effect both from the standpoint of digestibility and content of net energy. Schnepf (9, 10) fed silage preserved with mineral acids to sheep. He reported that the silage caused a negative calcium and phosphorus balance and that the addition of dry hay or of a mineral supplement had a favorable effect although a positive balance was not restored in every case.

These reports demonstrate the desirability of neutralizing A.I.V. grass silage by the addition to the ration of either basic salts or roughages with a high basic surplus. The present paper deals with the effect of feeding phosphoric acid grass silage on the acid-base balance of dairy cows and represents a companion study to one dealing with the chemical changes in this type of silage (Pagé and Maynard, 6). The fact that phosphoric acid is normally concerned in the acid-base regulation of the organism and that phosphorus is an essential mineral, suggested that this acid might cause lesser physiological disturbances than the stronger acids used in preserving silage by the A.I.V. process. Three separate experiments are here reported.

#### EXPERIMENT I

Four animals well along in lactation were selected for this first study. During a four week preliminary period, the cows were fed corn silage with mixed hay and concentrates. The concentrate mixture contained 18 per cent of protein and included one per cent of bone meal. The mixed hay was composed of timothy, red top, quack grass, alfalfa, blue grass and red clover. The corn silage was from a crop that was well eared and at a medium stage of maturity. Following the preliminary period, the cows were placed for six weeks on a ration of phosphoric acid silage and grain. This silage was preserved with 27 pounds of 68 per cent phosphoric acid (food grade) per ton of green material. It had the following composition: grasses, 50 per cent; clovers, 25 per cent; alfalfa, 20 per cent; and weeds, 5 per cent.

During the preliminary period, blood was collected for analysis at the end of the 2nd, 3rd and 4th weeks, respectively. Twenty-four hour urine collections were made at the end of the 2nd and 3rd weeks. Five blood and urine collections were made during the period of acid silage feeding, namely, at the end of the 1st, 2nd, 3rd, 4th and 6th weeks.

The CO<sub>2</sub> combining capacity of the blood plasma was determined with a Van Slyke manometric blood gas apparatus (7). The pH of the urine was measured with the quinhydrone electrode, the fixed CO<sub>2</sub> with the Van Slyke

manometric blood gas apparatus and the ammonia nitrogen by the Folin aeration method as modified by Steele (11).

The results of these analyses for Experiment I are summarized in table 1. Clearly, there was no change in the pH of the urine as a result of feeding the phosphoric acid silage. While the fixed CO<sub>2</sub> in the urine was lower in every case during the acid silage period, the values during the latter period did not reach a sufficiently low level to indicate any tendency to acidosis. Clearly, the values for NH<sub>3</sub>-nitrogen do not furnish any evidence of acidosis since they were actually lower during the silage period. It is also clear that the blood CO<sub>2</sub> combining capacity was not significantly altered. Thus all of the data in table 1 are in agreement in indicating that there was no tendency to acidosis when the phosphoric acid silage was fed. It should finally be stated that no detrimental effect followed the period of acid silage feeding with respect to weight, general appearance and milk production of the animals.

#### EXPERIMENT II

Four cows in heavy milk production were selected for this experiment. They were fed a ration containing phosphoric acid grass silage, mixed hay and grain during one period of six weeks and a ration containing molasses silage, mixed hay and grain during another period of similar length. The grain mixture, mixed hay and acid silage were of the same composition as in the first experiment. The molasses silage was slightly higher in legumes than the acid silage, its approximate composition being as follows: grasses, 35 per cent; clovers, 40 per cent; alfalfa, 20 per cent; and weeds, 5 per cent.

Urine was collected over a twenty-four hour period at the end of the 2nd and 6th weeks of the first period and at the end of the 1st, 2nd, 4th and 6th weeks of the second period. Blood samples were taken for the 3rd, 4th and 6th weeks of the first period and for the 1st, 2nd, 4th and 6th weeks of the second period. The analytical results of this experiment are summarized in table 2. These data clearly show that the pH of the urine was not changed as a result of feeding phosphoric acid silage. The changes which occurred in the fixed CO<sub>2</sub> and NH<sub>3</sub>-Nitrogen of the urine are not consistent nor significant enough to indicate a condition of acidosis. Likewise, the alkaline reserves of the blood plasma show no significant changes from one period to the other. It may be concluded that no consistent evidence is presented in table 2 indicating that the feeding of phosphoric acid silage resulted in any tendency to acidosis, compared to the feeding of molasses silage.

#### EXPERIMENT III

In this last experiment, the phosphoric acid grass silage was submitted to a somewhat more crucial test in that the crop thus preserved contained only traces of legumes and the grain mixture fed with it in two of the four cases contained no neutralizing agent.

TABLE I  
*Mean urine and blood values, Experiment I*

Cow	Urine values				Blood plasma CO <sub>2</sub> combining capacity, volume %		
	pH	Fixed CO <sub>2</sub> , mM (24 hours)	NH <sub>3</sub> -Nitrogen, mg. (24 hours)	H <sub>3</sub> PO <sub>4</sub> period	Prelin. period	H <sub>3</sub> PO <sub>4</sub> period	Prelin. period
Prelim. period	H <sub>3</sub> PO <sub>4</sub> period	Prelin. period	H <sub>3</sub> PO <sub>4</sub> period	Prelin. period	H <sub>3</sub> PO <sub>4</sub> period	Prelin. period	H <sub>3</sub> PO <sub>4</sub> period
1	8.3	8.3	1607	762	323	146	69.6
2	8.5	8.2	2207	814	303	269	64.5
3	8.4	8.2	1782	505	209	90	65.1
4	8.3	8.2	1691	854	281	149	74.2

TABLE 2  
*Mean urine and blood values, Experiment II*

Cow	Urine values				Blood plasma CO <sub>2</sub> combining capacity, volume %	
	pH	Fixed CO <sub>2</sub> , mM (24 hours)		NH <sub>3</sub> -Nitrogen, mg. (24 hours)	H <sub>2</sub> PO <sub>4</sub> silage period	Molasses silage period
1	8.3	8.1	449	257	236	608
2	8.3	8.3	994	874	606	66.9
3	7.9	8.2	915	538	122	72.9
4	8.3	8.3	1354	1542	439	207
					327	61.5
						67.5
						67.3
						72.8

Four cows, either dry or well along in lactation, were selected for this study. These animals were sterile but in thrifty condition. All experimental animals received corn silage and mixed hay for four weeks. The corn silage was from a well eared crop at a medium stage of maturity. The mixed hay, which was early cut and well cured, contained about 50 per cent of legumes. During this preliminary period, twenty-four hour urine collections were made at the end of the 2nd and 4th weeks. Blood samples were taken at the end of the 3rd and 4th weeks.

The cows were then placed on phosphoric acid silage alone for the next four weeks. This silage was made from a timothy crop, early bloom, which contained a small amount of other grasses and only a trace of legumes. It was preserved with 20 pounds of 68 per cent phosphoric acid (food grade) per ton of green material. At the end of this time, two of the animals received some grain in addition to the silage during the next four weeks, while the other two remained on phosphoric acid silage as the only feed. During this period, with the exceptions noted later, urine collections were made at the end of the 2nd, 7th and 9th weeks. Blood samples were taken at the end of the 2nd, 4th and 7th weeks.

During a further period of five weeks, the acid silage was neutralized as follows: nine ounces of ground limestone per 100 pounds of silage were given to one of the cows which had been receiving silage only, and also to one of the cows on the silage and grain ration. The other two cows received mixed hay in addition to their previous ration: cow No. 2 received an average of 5 pounds of hay daily and cow No. 4 received 4.5 pounds. The mixed hay was early cut and well cured. It contained clover, alfalfa, timothy and other grasses. The legumes constituted about fifty per cent of the crop. Urine and blood samples were taken after the cows had been on the modified rations for two weeks and again at the end of the fifth week.

When the cows were put on phosphoric acid grass silage as the only feed, three of the milking animals dried up quickly. Two of the four animals lost flesh and failed to eat properly. Only one cow (No. 4) remained in good condition throughout the experiment. All showed some improvement when either mixed hay or limestone was added to their diet. Cow No. 4 showed an increase in appetite, in that she cleaned up her feed more readily.

The results of the blood and urine analyses are summarized in table 3. The samples collected during the period of acid silage feeding were not entirely satisfactory. One of the cows (No. 1) went off feed during this period and was put on a ration containing a variety of feeds for several weeks. Table 3 includes only her last set of values in this period, obtained after she had been back on acid grass silage and grain for two weeks. One set of values for cow No. 4, obtained during the 7th week had to be discarded because it was found that she had been stealing hay from a neighboring animal. This point will be taken up later. In the case of animal No. 2, only

TABLE 3  
Mean urine and blood values, Experiment III

Cow	Urine values						Blood plasma volume %		
	pH		Fixed CO <sub>2</sub> , mM (24 hours)		NH <sub>3</sub> -Nitrogen, mg. (24 hours)		Period 3 H <sub>2</sub> PO <sub>4</sub> silage plus lime or hay	Period 3 H <sub>2</sub> PO <sub>4</sub> silage plus lime or hay	Period 3 H <sub>2</sub> PO <sub>4</sub> silage plus lime or hay
	Period 1 Herd ration	Period 2 H <sub>2</sub> PO <sub>4</sub> silage	Period 1 Herd ration	Period 2 H <sub>2</sub> PO <sub>4</sub> silage	Period 1 Herd ration	Period 2 H <sub>2</sub> PO <sub>4</sub> silage			
1	8.5	5.9	7.8 <sup>a</sup>	17.08	8	8.89 <sup>a</sup>	243	227.9	276 <sup>a</sup>
2	8.1	5.7	8.3 <sup>b</sup>	10.99	3	4.90 <sup>b</sup>	199	441	57.5
3	8.4	5.6	8.6 <sup>a</sup>	10.40	2	10.93 <sup>a</sup>	195	274.8	61.6
4	8.5	5.5	8.3 <sup>b</sup>	7.40	1	5.68 <sup>b</sup>	401	718	55.8

<sup>a</sup> Silage plus limestone.

<sup>b</sup> Silage plus hay.

three of the seven blood samples could be analyzed because the other four hemolyzed on centrifugation. Her blood appeared to be abnormally high in cell volume. Unfortunately, it was impossible at the time to investigate this condition further.

In spite of these shortcomings, a rather clear picture is presented in table 3. It is noted that when the cows were changed from the herd ration to the phosphoric acid grass silage alone, there was a marked drop in the pH of the urine, a nearly total disappearance of the fixed CO<sub>2</sub>, and a very marked rise in the urinary ammonia. These changes were not modified by the addition of grain which was made in the case of two of the cows during the last five weeks of period 2. The urinary values for period 3 show that when the phosphoric acid grass silage was supplemented by either limestone or hay, in the amounts previously stated, the tendency to acidosis was entirely overcome. The values for all urinary constituents became similar to those obtained during period 1.

An interesting bit of evidence relative to the effectiveness of hay in correcting the acid condition was obtained as a result of an unplanned consumption of hay by cow No. 4 in period 2. The first values obtained on this cow were in agreement with those shown by the other animals. Her next values revealed a marked improvement as regards the condition of acidosis. She had been observed on one or two occasions to be stealing hay from a neighboring cow not on the experiment. This hay was of the same composition as that fed during the third period and contained approximately 50 per cent of legumes, mostly clover. When a partition was put in to prevent hay consumption by cow No. 4, the succeeding values revealed the same state of acidosis as shown by the other cows.

In contrast to the above changes which occurred in the urine, it is noted in the last three columns of table 3 that the blood CO<sub>2</sub> combining capacity was not significantly altered by the feeding of phosphoric acid grass silage. Thus, it is apparent that the physiological changes reflected so clearly in the urine did not affect the alkaline reserve of the blood. This might lead to the conclusion that there was no essential disturbance of the acid-base balance were it not for the fact, as mentioned earlier, that three of the cows were in poor condition as a result of feeding the phosphoric acid grass silage alone. Their condition was markedly improved when urinary values were brought back to normal by the feeding of either hay or limestone.

#### DISCUSSION

While the alkaline reserve of the blood was not affected by feeding the acid grass silage as the sole ration, the effect on the condition of the cows, coupled with the marked changes in urine, suggests that grass silage ensiled with twenty pounds of phosphoric acid per ton is not a satisfactory sole ration for dairy cows but that some neutralizing agent should be included.

While it is generally assumed that urinary acidosis is of little physiological importance as long as the alkaline reserve of the blood plasma is maintained at a normal level, the present experiments suggest that a normal alkaline reserve is not in itself a sufficient criterion of a healthy condition. It is possible that more importance should be given to the urine picture than has usually been the case. Since cow No. 4 remained in good condition in spite of the acidosis revealed by the urine picture, it is also possible that some other factor is concerned for which no suitable chemical determinations have been made.

Some comment should be made on the fact that acid silage and grain proved satisfactory in Experiment I whereas it did not in Experiment III. In the first experiment, the silage contained a large amount of legumes and the grain mixture contained bone meal. In the third experiment, the silage was undoubtedly lower in lime and other basic constituents for the reason that it consisted almost entirely of grasses. Further, the grain mixture contained no bone meal. These differences in the rations fed might explain the differences in the results obtained. In the last experiment, the addition of grain following a period when the silage was the sole ration did not improve the condition of the animals. This is evidence that the condition was not due simply to a lack of a sufficient feed intake. On the other hand, since the addition of limestone alone was beneficial, it is apparent that the source of the trouble lay with the acidity of the silage.

#### SUMMARY

Three experiments involving a total of twelve animals are reported in which the changes in the acid-base relations in the blood and urine were studied with cows receiving phosphoric acid silage with and without hay or limestone. No changes in the blood or urine were observed, as compared with the data on a ration including corn and molasses silage, when the phosphoric acid silage was supplemented with either hay or limestone. The feeding of phosphoric acid grass silage alone resulted in marked urinary changes indicating acidosis but was without effect on the alkaline reserve of the blood. Three of the four animals developed an unthrifty condition when fed the silage alone. This condition was relieved and the urine values were brought back to normal by the addition of either hay or limestone. It is concluded that the phosphoric acid grass silage is a satisfactory feed for dairy cows when it is supplemented with hay containing an appreciable amount of legume or with limestone.

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## BROOMCORN SILAGE FOR DAIRY CATTLE

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Illinois produced 29,000 acres, or approximately 13 per cent, of the 223,000 acres of broomcorn harvested in the United States in 1939. The acre yield is somewhat greater in Illinois than in the other five principal producing states which accounts for the fact that during the period 1928-1939 from 20 to 25 per cent of the harvested crop was grown in this state (1).

In harvesting broomcorn, the brush is removed by hand cutting shortly after it has emerged from the enveloping sheath at the top of the plant. At this stage the plant is still green, that is, it contains a large amount of moisture. As a rule, the brush is the only portion of the crop used and the remaining stalks, which may yield several thousand pounds of dry matter per acre, are plowed under. In some cases the crop, or a portion of it, is permitted to stand until maturity for the production of seed. It appears that the principal reasons the stalks are not used as forage as soon as the harvesting of the brush is completed are that the crop is unpalatable and supposedly poisonous to livestock.

Very few reports of experimental studies of the value or possibilities of broomcorn as a forage crop are to be found in the literature. Dowell and Friedemann (2) of Oklahoma conducted an investigation of the composition and digestibility of the immature broomcorn seed which is removed immediately after harvesting the brush. In this investigation they also studied the composition of the stover and the composition and digestibility of silage made from the green broomcorn plant. They report that broomcorn seed is less valuable as a feedstuff than the grain sorghum although the digestion coefficients of the two compare favorably. They report further that broomcorn silage kept in good condition, had a pleasant odor, was eaten with relish by sheep, and the digestibility of the dry matter of the silage was 51.5 per cent. They found that the amount of prussic acid in a sample of the whole plant harvested July 6th was 0.0098 per cent and the amount of tannin in the whole plant was about 0.2 per cent and in the seed 0.58 to 0.86 per cent.

The objects of the investigation reported herewith were: (a) to determine the yields of dry matter in the stalk portion of the broomcorn plant; (b) to find a suitable method for the preservation of the stalks as silage; and (c) to study the feeding value of the silage.

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#### EXPERIMENTAL PROCEDURE

Seed of the two varieties of broomcorn commonly grown in Illinois, namely, Black Jap and White Italian, was drilled in rows 3.5 feet apart in May, 1938. Adjoining the broomcorn plot, were plots of Orange sorgo and hybrid corn. The broomcorn was cultivated in the same manner as corn.

Beginning on August 9 and at intervals up to September 20, the crop from measured lengths of row was harvested and the yields of fresh matter and of dry matter were determined. The yields for August 9 and August 13 include the brush because on these dates many of the heads (brush) had not yet emerged and accurate separation was impossible.

Portions of the crop of each variety were ensiled in small containers at approximately two-week intervals, beginning on August 9. The containers were of galvanized iron construction and each held about 100 pounds of crop. The crop ensiled on August 9 included the brush but at later dates the brush was removed before passing the stalks through the silo-filling machine. The cut material was packed tightly by tramping.

The Black Jap variety reached about the right stage for brush harvest on August 22 while the White Italian variety appeared to need 7 to 10 days longer to reach this stage. A quantity of each variety was harvested on August 22, the brush removed, the stalks chopped by means of a silo filler and the cut material collected on a wagon. Small containers were filled as on previous dates. After weighing the remainder of the cut material, it was sprayed and thoroughly mixed with cane feeding molasses at the rate of 100 pounds of molasses to a ton of crop. Before application the molasses was diluted with an equal weight of water. A wood silo 4 feet in diameter and 10 feet deep was filled with the silage material of the Black Jap variety and another silo of the same size with White Italian. In both the small containers and wood silos the silage was covered with heavy asphalt roofing material and weighted with ground limestone.

The small containers were opened eight or nine months after filling. In most cases samples were taken for determination of dry matter and water-soluble acidity, but in some of the containers the silage was completely spoiled and no samples were taken. The wood silos were opened 15 to 16 months after filling and the silage fed to four Holstein cows during a double-reversal feeding trial. The experimental periods were four weeks in length with a one-week preliminary period and a one-week transition period. Comparison of broomcorn silage was made with corn silage.

#### EXPERIMENTAL RESULTS

It was found that both varieties of broomcorn gave large yields of fresh matter and of dry matter (table 1). The yields increased rapidly and continued to increase in the unharvested portion of the crop even after the usual stage for brush harvest. The yield of the Black Jap variety on Sep-

tember 20 was less than for the previous harvest date, a difference probably accounted for by the extensive breaking down of the plants brought about by the development of the heavy seed crop on the brush.

The White Italian is a larger-growing variety than the Black Jap, with larger and taller stalks. Twenty-five consecutive plants in a row of each variety were measured on September 20. The average length of the 25 harvested plants of the Black Jap variety was 125.6 inches and of the White Italian, 150.6 inches.

TABLE 1  
*Yields of fresh matter and of dry matter in broomcorn*

Date of harvest	Variety	Dry matter in crop	Yield of crop per acre <sup>1</sup>	
			Fresh matter	Dry matter
8-9-'38	Black Jap	19.50	26390	5146
8-9-'38	White Italian	16.70	28650	4875
8-13-'38	Black Jap	22.38	31125	6966
8-13-'38	White Italian	22.57	39000	8802
8-22-'38	Black Jap	28.51	23374	5589
8-22-'38	White Italian	27.92	31668	7368
8-27-'38	Black Jap	30.05	26200	7873
8-27-'38	White Italian	27.78	33500	9306
9-20-'38	Black Jap	32.31	20858	6739
9-20-'38	White Italian	34.36	28146	9671

<sup>1</sup> The yields reported for the harvests of August 9 and August 13 are for the entire harvested crop including the brush. The yields given for the other dates are for the harvested plants after removal of the brush.

The yields of the stalk portion of the broomcorn crop at the time the brush would normally be harvested, which in 1938 was the latter part of August, were nearly or fully as large as those of hybrid corn and Orange sorgo grown on adjoining plots. Several plots of hybrid corn harvested September 12 to 16, yielded from 7000 to 8000 pounds of dry matter per acre, while Orange sorgo harvested September 20 yielded 9070 pounds.

All of the lots of broomcorn ensiled without molasses were either completely spoiled or in very poor condition when the silos were opened (table 2). It appeared that insufficient acid was produced to bring about preservation. Also, broomcorn ensiled after the usual brush-harvest stage (August 22 to 31 in this investigation) seemed to lack sufficient amounts of moisture. While these results are only preliminary, they point strongly to the conclusion that for best results broomcorn stalks should be ensiled immediately following brush harvest and that liberal amounts of molasses or other preservative should be added.

The broomcorn ensiled in the wood silos on August 22 (Silos 2 and 3, Table 2) kept well, and had a good green color, strongly acid odor, and pleasant aroma. Several days were required for the cows to become accus-

TABLE 2  
*Results obtained in ensiling broomcorn at different stages of development*

Silo No.	Date filled	Date emptied	Crop	Treatment	Dry matter in crop as ensiled		Lactic acid in silage		Condition of silage
					%	Dry matter in silage as emptied	Fresh silage	Dry matter of silage	
376	Aug. 9	May 2	Black Jap	None	19.50	14.11	.051	.36	Yellow; bad odor
424	Aug. 9	May 2	White Italian	None	16.70	10.17	.104	1.02	Very wet
444	Aug. 9	May 2	{ Broomcorn, 50% Soybeans, 50%	None	{ 18.10 18.92	13.27	.276	2.08	Too much moisture
449	Aug. 22	May 9	Black Jap	None	29.51	26.67	.025	.09	Too dry
628	Aug. 22	May 9	White Italian	None	27.92	22.95	.025	.11	Moldy
3	Aug. 22	Dec. 11	Black Jap	Molasses, 5% Molasses, 5% 1	28.51	30.65	1.63	5.32	Good
2	Aug. 22	Nov. 13	White Italian	Molasses, 5% Molasses, 5% 1	27.92	29.38	1.87	6.38	Good
17	Sept. 6	May 2	Black Jap	Molasses, 5% Molasses, 5% 1	29.75	22.64	—	—	Dry; moldy
16	Sept. 6	May 2	White Italian	Molasses, 5% Molasses, 5% 1	29.70	21.38	.079	.37	Dry; moldy
385	Sept. 6	May 2	{ White Italian, 50% Soybeans 50%	Water, 10%	{ 29.70 28.29	18.93	.103	.54	Moldy
18	Sept. 6	May 2	{ White Italian, 50% Soybeans, 50%	Molasses, 5% 1	{ 29.70 28.29	25.09	.896	3.57	Fair; acid odor
21	Sept. 20	May 9	Broomcorn	Molasses, 5% Molasses, 5% 1	32.31	24.03	—	—	Very moldy
22	Sept. 20	May 9	Broomcorn	Molasses, 5% Molasses, 5% 1	34.36	29.94	—	—	Very moldy

<sup>1</sup> Molasses was diluted with equal weight of water before application.

tomed to the silage and at no time during the experimental periods did they consume as much broomcorn silage as corn silage. Broomcorn stalks are hard and rigid and in the ensiling process the knives did not cut off all the stalks squarely but splintered many of them. These pieces were from two to five inches in length and remained hard and sharp even after 15 months



FIG. 1. Broomcorn Grown for Silage Investigation. Two rows of broomcorn photographed on August 20. At that time the heads (brush) of the Black Jap variety (left) were almost ready for brush harvest, while part of the heads (brush) of the White Italian variety (right) were just emerging from the enveloping sheath. Height of man, 6 feet.

in the silo. The refused portion of the silage, which amounted to 17.8 per cent of the silage fed, consisted mainly of these sharp pieces. It is likely that fine chopping by a machine with sharp knives would reduce the amount of silage refused.

The broomcorn silage seemed to have good nutritive value, as judged by its chemical composition (table 3) and by the milk production of 4 cows to which it was fed (table 4). The production of the cows declined more rapidly during the broomcorn silage periods than during the corn silage periods but this is accounted for in part at least by a lower feed intake when broomcorn silage was fed. So far as could be observed, the consumption of broomcorn silage had no harmful effects upon the cows.

The broomcorn silage was found to be high in fiber (table 3).

TABLE 3  
*Composition of broomcorn silage used in feeding trial*

Variety of broomcorn	Dry matter	Ash	Total protein	Ether extract	Crude fiber	Nitrogen-free extract
	%	%	%	%	%	%
<b>Fresh basis</b>						
Black Jap .....	30.65	2.50	1.41	0.44	10.74	15.56
White Italian .....	29.38	2.11	1.34	0.42	10.98	14.53
<b>Dry basis</b>						
Black Jap .....	100.00	8.15	4.61	1.42	35.04	50.78
White Italian .....	100.00	7.18	4.55	1.41	37.36	49.50

TABLE 4  
*Summary of results of feeding broomcorn silage in comparison with corn silage to dairy cows*

Kind of silage	No. of cows	Silage fed daily	Silageorts	Feed consumed daily per cow			Test of milk	Milk yield daily per cow <sup>1</sup>
				Silage	Hay	Grain		
Broomcorn .....	4	lbs.	lbs.	lbs.	lbs.	lbs.	%	lbs.
Corn .....	4	21.1	3.8	17.3	22.0	13.9 <sup>2</sup>	3.61	34.4

<sup>1</sup> Milk energy in terms of 4% milk computed according to the formula .4 × milk (in pounds) + 15 × fat (in pounds).

<sup>2</sup> Includes 3 pounds of dried beet pulp.

#### SUMMARY

A small-scale investigation of the possibilities of using broomcorn for silage was conducted. Two varieties of broomcorn, Black Jap and White Italian, produced large yields of dry matter per acre, the amounts in the stalk portion of the plant at the usual brush-harvest stage being as large as the amounts in hybrid corn and in Orange sorgo harvested for silage. The White Italian is a larger growing variety than the Black Jap and gave larger yields of dry matter.

Broomcorn was ensiled at different stages of development. Best results were obtained in the case of broomcorn stalks ensiled at the usual stage for brush harvest.

Silage very low in acidity and with poor keeping qualities was found in all silos except those in which the cut crop was treated with molasses at the rate of 100 pounds per ton.

Broomcorn silage was found to be high in fiber content and hard splintered pieces several inches in length were refused by dairy cows. After becoming accustomed to the new feed, the cows ate the silage readily though

not as freely as when corn silage was fed, and produced only slightly smaller amounts of milk than during the corn silage periods.

The results obtained justify the conclusion that it is possible to make large amounts of silage having good keeping qualities and fair feeding value from the stalks of broomeorn, the portion of the plant now rarely utilized in any way except for plowing under.

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## AGE, LIVE WEIGHT AND MILK-ENERGY YIELD IN ILLINOIS COWS

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In a previous article it was mentioned (1, footnote 6) that a plan of using live weight in connection with Dairy Herd Improvement Association records was under trial. This paper reports some of the results of that trial, dealing with all the Holstein (957) and Jersey (195) records available covering the first 8 monthly tests of the lactation without interruption. The records were all made under farm conditions, milking twice daily. It is impossible to deal with the records beyond the 8th month without in many cases encountering serious disturbances associated with advanced pregnancy and with management practice. Comparisons are simplified and biological meanings are clarified by dealing with the first 8 months of lactation (when the usual calving interval is 12 months) as compared with trying to deal with the whole lactation, or with fiscal year records. This fact was recognized by Gowen (2) as early as 1920, but the merits and advantages of the system are not yet generally appreciated.

### ESTIMATE OF LIVE WEIGHT

Live weight of the individual cows was determined by use of a chest-girth live-weight tape sold by the New York Farm Bureau Federation, Ithaca, New York. The determination was made uniformly for each cow at the first visit of the tester after the cow calved, that is, within the first 30 days following parturition.

The tape used is marked at one centimeter intervals and each mark is labeled with a corresponding live weight in pounds. The following figures, taken from the tape, cover the range of size encountered in the present trial:

Chest girth, cm.	140	150	160	170	180	190	200	210	220
Live weight, lbs.	507	617	750	908	1085	1261	1437	1614	1790

### AGE, LIVE WEIGHT AND YIELD

Only 255 of the Holstein records and 163 of the Jersey records include age of cow. The relation of age to live weight is shown graphically in figure 1; that of age to FCM,<sup>2</sup> in figure 2; that of live weight to FCM, in figure 3.

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<sup>1</sup> Acknowledgment is made to the several testers who secured the live weights and reported the records of the cows on which this paper is based.

<sup>2</sup> Symbols are used in this paper as follows:

FCM = milk-energy yield per day, pounds of 4% milk. (Calories of milk energy = 340 FCM.)

Table 1 proceeds to a more critical analysis of the separate and relative

TABLE 1

*Influence of live weight (W) and age (A) on milk-energy yield (FCM) for cows under 7 years of age (Holstein, n = 199; Jersey, n = 140)*

Breed	Partial lactation	Mean FCM	FCM = BW + DA		FCM = a + bW + dA			$\sigma_{wb}/\sigma_A d$
			BW	DA	a	1000b	d	
Holstein	1 mo.	37.7	30.7	7.0	-1.11	26.33	1.76	1.7
Jersey	1 mo.	36.1	35.6	0.5	6.92	32.56	0.70	3.8
Holstein	2 mos.	36.4	29.5	6.9	1.06	23.44	1.78	1.5
Jersey	2 mos.	35.3	35.5	-0.2	5.28	35.06	0.35	8.1
Holstein	3 mos.	35.0	28.5	6.4	3.83	20.15	1.71	1.3
Jersey	3 mos.	34.3	34.0	0.3	6.08	31.89	0.57	4.5
Holstein	4 mos.	33.7	28.2	5.4	4.51	19.29	1.47	1.5
Jersey	4 mos.	33.3	32.8	0.5	4.99	32.12	0.55	4.7
Holstein	5 mos.	32.6	27.7	4.8	5.43	18.10	1.34	1.5
Jersey	5 mos.	32.4	31.9	0.5	5.56	30.19	0.57	4.3
Holstein	6 mos.	31.5	27.2	4.2	7.23	16.09	1.22	1.5
Jersey	6 mos.	31.4	31.1	0.3	5.20	29.72	0.50	4.8
Holstein	7 mos.	30.4	26.8	3.5	8.54	14.53	1.09	1.5
Jersey	7 mos.	30.5	30.2	0.2	5.95	27.54	0.53	4.2
Holstein	8 mos.	29.2	26.1	2.9	9.89	12.84	0.96	1.5
Jersey	8 mos.	29.5	29.9	-0.5	6.48	26.37	0.37	5.8

$\bar{W}$  = mean W = 1211 pounds for Holstein cows; 822 pounds for Jersey cows.

$\bar{A}$  = mean A = 3.93 years for Holstein cows; 3.51 years for Jersey cows.

$\sigma_w$  = 166.5 pounds for Holstein cows; 107.3 pounds for Jersey cows.

$\sigma_A$  = 1.481 years for Holstein cows; 1.328 years for Jersey cows.

influence of age and live weight on FCM, for partial lactations of 1, 2, 3, 4, 5, 6, 7, and 8 months. In comparing the partial lactations of different lengths it should be borne in mind that differences may be affected by two factors: the effect of advance in lactation which may operate differently in different cows; the effect of summing or averaging, e.g., the 1-month partial lactation is a single one-day test while the 8-month partial lactation is an average of 8 one-day tests spaced one month apart. Table 1 deals with cows less than 7 years of age in order to have substantially linear regression of FCM on age.

The last 4 columns of table 1 give the results of fitting the equation  $FCM = a + bW + dA$  to the individual observations, that is, the observations are described by a plane which pivots at a point located at the mean of FCM, the mean of W, and the mean of A. The constant b in the equation describes

W = live weight of cow, pounds (determined within the first 30 days after calving)

A = age of cow at calving, years

n = number of cows or records

$\sigma$  = standard deviation

V = variance =  $\frac{\text{sum of squares of deviations from mean}}{\text{degrees of freedom}} = \sigma^2$

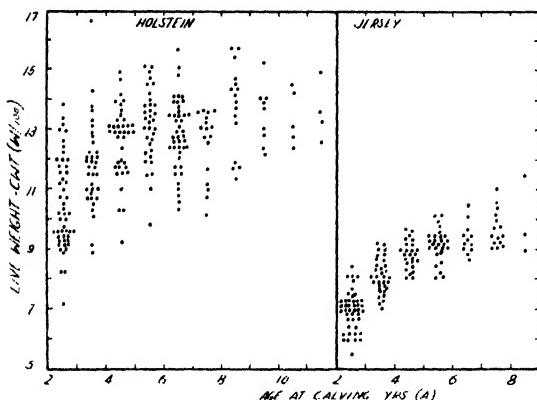


FIG. 1. Age and live weight. Omits 5 Holstein records above 11 years and 5 Jersey records above 8 years.

the slope which the plane takes under the influence of W independent of A; and the constant d describes the slope which the plane takes under the influence of A independent of W. The constant a is the mean FCM minus the sum of b times mean W and d times mean A.

A proper comparison of b and d as measures, respectively, of the influence of live weight and age on FCM yield requires weighting by the standard deviations (cf. 3, page 277). The last column of table 1 gives this weighted comparison and according to this point of view, it appears that weight is about 1.5 times as influential as age in the Holstein records, and about 5 times as influential in the Jersey records, in determining milk-energy yield.

Figure 6 gives a graphic representation of the planes, the equations of which are given in table 1, for the 1-month and 8-month partial lactations, and serves to give perhaps a more comprehensible picture of the relative influence of age and weight on milk-energy yield. For example in the Jersey 8-month record, as age increases by 5 years, there is an increase of 1.7 in FCM independent of weight; but as weight increases by 600 pounds, there is an increase of 15.8 in FCM independent of age. Clearly, live weight rather than age is the dominating influence on milk-energy yield.

Table 1 gives also the constants resulting from fitting the equation  $FCM = BW + DA$ . This equation, like the one above, describes the observations by a plane but in this case the plane pivots at the point of origin (instead of the point of means), that is,  $FCM = 0$  when weight and age are each zero. It assumes that FCM is a multiple of W plus a multiple of A. In case of the Jersey records, the multiple of W accounts almost entirely for FCM, leaving practically nothing left to be accounted for as a multiple of age. A similar condition prevails in the Holstein records, although slightly less extreme in the domination of live weight over age.

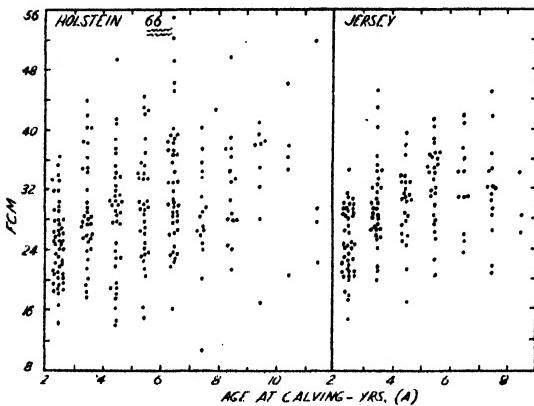


FIG. 2. Age and FCM for partial lactation of 8 months. Omits same ages as figure 1. Compare figure 4.

The above relations have very important significance in connection with the biological soundness of the much-used system of correcting milk records for age of cow. It is clear that correction for age is justifiable only in case the initial live weight is unknown, and then only insofar as weight is related to age and correctly portrayed by the particular set of age-correction factors that may be used. Age correction is an indirect allowance for initial live weight. It is biologically unsound and should be superseded by a system based on initial live weight, which can be readily used to eliminate the indirect influence of age as will be developed in the next section.

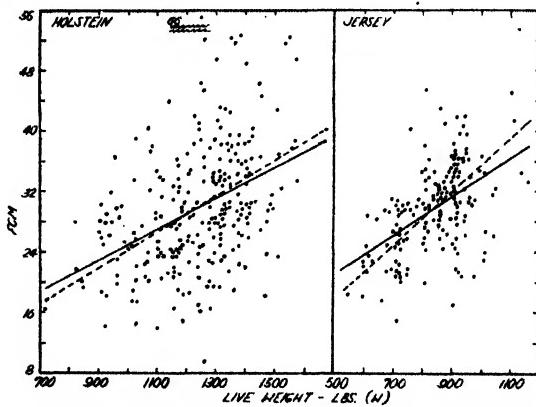


FIG. 3. Live weight and FCM for partial lactation of 8 months (ages known). The solid lines are fitted by least squares: Holstein,  $FCM = 4.6 + .0205W$ ; Jersey,  $FCM = 8.1 + .0257W$ . The broken lines cut the origin and means: Holstein,  $FCM = .0242W$ ; Jersey,  $FCM = .0353W$ . Power equation,  $FCM = bWe^c$ , curve not shown: Holstein,  $c = .94$ ; Jersey,  $c = .87$ . Compare figure 5.

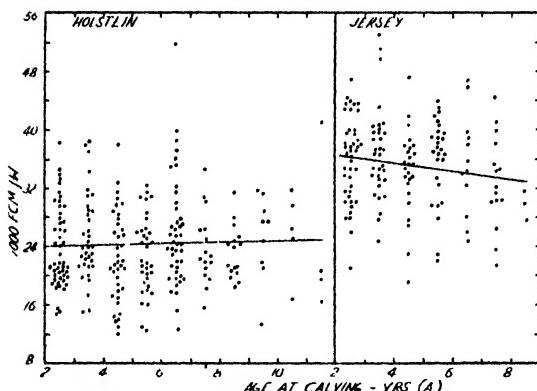


FIG. 4. Age and FCM/W for partial lactation of 8 months. Omits same ages as figure 1. Straight lines are fitted, including all ages, by least squares: Holstein, 1000 FCM/W =  $23.7 + .12A$ ; Jersey, 1000 FCM/W =  $37.4 - .58A$ . Compare figure 2.

#### AGE AND FCM, W

The results (table 1) of fitting the equation,  $FCM = BW + DA$ , show that D is small and suggest that, so far as age is concerned,  $FCM = BW$ , practically. Hence,  $FCM \propto B$ , a constant so far as age is concerned, and a simple rational way to eliminate change in yield with age is to deal with  $FCM/W$ . Whether  $FCM/W$  is in fact independent of age may be tested directly by an analysis of variance (cf. 3, page 182). The test is to determine whether the variance of  $FCM/W$  is significantly greater between age groups than it is within age groups. If it is significantly greater, age differences must be considered; if it is not significantly greater, age differences may be disregarded. The results are given in table 2.

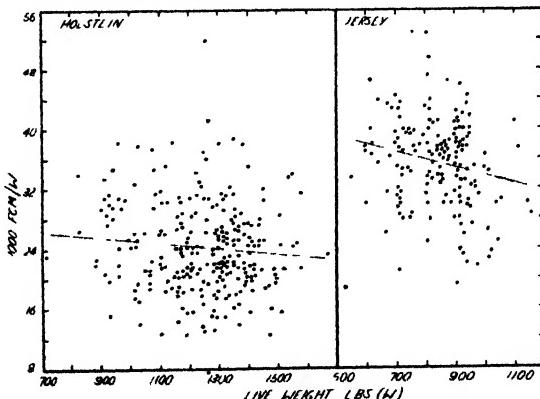


FIG. 5. Live weight and FCM/W for partial lactations of 8 months (ages known). Straight lines are fitted by least squares: Holstein, 1000 FCM/W =  $29.0 - .0038W$ ; Jersey, 1000 FCM/W =  $44.0 - .0101W$ . Compare figure 3.

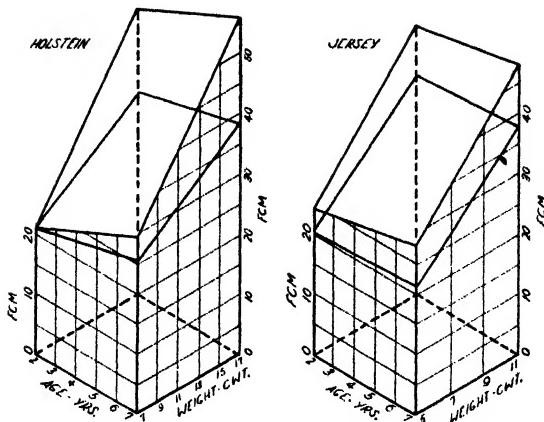


FIG. 6. Age, live weight and FCM for partial lactations of 1 month (upper plane) and 8 months (lower plane). Constants of the equations of the planes,  $FCM = a + bW + dA$ , are given in table 1.

TABLE 2  
*Analysis of variance, records classified by age  
(1 year intervals, age classes, 2-13 years)*

	1000 FCM/W for partial lactation of							
	1 mo.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.	7 mos.	8 mos.
Holstein, n = 255								
V, between groups	121.7	124.7	100.3	84.4	73.3	59.5	55.3	44.6
V, within groups	79.7	73.1	66.6	58.5	50.9	46.0	42.6	37.6
F <sup>a</sup>	1.53	1.71	1.51	1.44	1.44	1.29	1.30	1.19
Regression <sup>b</sup>	+ 0.40	+ 0.39	+ 0.36	+ 0.35	+ 0.28	+ 0.23	+ 0.18	+ 0.12
Jersey, n = 163								
V, between groups	111.3	89.2	87.1	84.8	79.9	69.4	60.0	53.0
V, within groups	74.1	53.8	45.4	44.2	41.3	38.5	37.3	36.5
F <sup>a</sup>	1.50	1.66	1.92*	1.92*	1.93*	1.80	1.61	1.45
Regression <sup>b</sup>	- 0.55	- 0.50	- 0.53	- 0.52	- 0.53	- 0.54	- 0.54	- 0.58

\* Test of significance. \* Significant at the 5 per cent level.

<sup>b</sup> Change in 1000 FCM/W for an increase of 1 year in age.

Taking the Holstein 8-month records in table 2 as an example, the ratio of the larger variance to the smaller, designated as  $F$ , is 1.19. The degrees of freedom are respectively 11 and 243. Reference to Snedecor's (3, page 184) table shows that in this case an  $F$  value of 1.83 is required at the 5 per cent level of significance. The  $F$  value, 1.19, is far below the 5 per cent level of significance and accordingly we may conclude there are no significant differences between age groups with respect to  $FCM/W$ . A similar conclusion applies to the Jersey 8-month records.

The regression of  $FCM/W$  on age, table 2, is positive in the Holstein records and negative in the Jersey records. This may be due to a breed

difference, but a more likely interpretation seems to be that the true regression in both breeds is zero. FCM/W may be used freely without regard to age of cow, and affords a simple rational solution of the "age-correction problem" which may be presumed to apply to dairy cows in general.

#### TWO RECORDS OF THE SAME COW

Among the records with age given only 12 Holstein cows and 1 Jersey have two records each. These records for the Holstein cows are shown in table 3. Age-corrected (A-C) FCM is based on the factors used by the Bureau of Dairy Industry in the proved sire work.

There are several points of interest in table 3. The variance between cows is much greater than that within cows for each of the attributes of lactation considered, *viz.* FCM, age-corrected FCM and FCM/W. With respect to FCM and age-corrected FCM cow No. 12 plays a prominent part as shown by the variance values (between cows) including and excluding this individual. With respect to FCM/W the variance values (between cows) are not very much affected by the presence or absence of cow No. 12.

TABLE 3

*Correlation between two records of the same cow with respect to FCM, age-corrected (A-C) FCM and FCM/W for partial lactation of 8 months*

Cow No.	Age		W		FCM		A-C FCM		1000 FCM/W	
	1	2	1	2	1	2	1	2	1	2
1	2	3	945	1224	31.9	42.0	38.6	46.3	33.8	34.3
2	2	3	1020	1166	33.6	36.4	40.7	40.1	32.9	31.2
3	3	4	1110	1074	24.2	24.1	26.7	25.0	21.8	22.4
4	3	4	1173	1496	21.3	31.7	23.5	32.8	18.2	21.2
5	4	5	926	979	27.9	30.2	28.9	30.4	30.1	30.8
6	4	5	1194	1415	30.6	29.0	31.7	29.2	25.6	20.5
7	4	5	1296	1208	31.4	26.5	32.5	26.7	24.2	21.9
8	4	5	1384	1243	28.9	23.3	29.9	23.5	20.9	18.7
9	5	6	1261	1402	35.4	36.1	35.6	36.1	28.1	25.7
10	6	7	1085	1120	37.6	33.8	37.6	34.0	31.7	30.2
11	6	7	1208	1279	28.8	26.9	28.8	27.1	23.8	21.0
12	12	13	1550	1544	52.3	51.8	57.8	58.5	33.7	33.5
Cows 1-11	$V_b$ , between cows . . .		40.83		64.49		54.00			
	$V_b$ , within cows . . .		13.63		11.46		2.95			
	$F_a$ . . .		3.00*		5.62**		18.28**			
	Intraclass correlation . . .		0.50*		0.70**		0.90**			
Cows 1-12	$V_b$ , between cows . . .		114.32		172.32		59.07			
	$V_b$ , within cows . . .		12.50		10.53		2.71			
	$F_a$ . . .		9.14**		16.36**		21.81**			
	Intraclass correlation . . .		0.80**		0.88**		0.91**			

\* Test of significance; \* Significant at the 5 per cent level; \*\* Significant at the 1 per cent level.

The intraclass correlation in table 3 is a measure of consistency in the magnitude of the two records with respect to the given attribute, assuming diversity exists between the individual cows. The records are most consis-

tently alike for the same cow and unlike for different cows in the case of FCM/W. This in itself need not necessarily mean that FCM/W is the best measure of lactation. The question turns too much on the true degree of likeness actually existing among the several cows involved. To measure likeness some quantitative criterion must be used and this necessitates the use of a philosophy of some sort.

The problem may be illustrated by considering the data for cow No. 12 in table 3. By the age-correction philosophy this cow is by far the best one of the lot. By the FCM/W philosophy she is distinguished from the others in being a large cow (1550 pounds) but not in any extraordinary dairy tendency or proclivity to lactation, being exceeded slightly by one and nearly equalled by 3 others in FCM/W. Cannot the biological aspects of milking capacity in dairy cattle be put on a more fundamental basis by dealing with size of cow and yield per unit size, rather than with yield alone or age-corrected yield? This raises the important practical and biological questions: Is FCM/W independent of W in these records? If not, can we expect to develop dairy cows to the point where FCM/W is independent of W, that is, to the point where milk-energy yield, or lactation work, is proportional to initial live weight?

#### LIVE WEIGHT AND FCM/W

Dealing first with the records where age is known, table 4 presents an analysis of variance to test the significance of differences between live weight groups with respect to FCM/W in a manner similar to that used in table 2 for age.

In the Holstein records, by the F test the differences between live weight groups with respect to FCM/W are below the 5 per cent level of significance for each of the 8 periods of partial lactation, 1 to 8 months. For the 1-month,

TABLE 4  
*Analysis of variance, records (age known) classified by live weight (100-pound intervals, weight classes 500-1600 pounds)*

	1000 FCM/W for partial lactation of							
	1 mo.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.	7 mos.	8 mos.
Holstein, n = 255								
V, between groups	48.9	29.1	35.2	38.5	37.3	37.8	39.9	45.6
V, within groups	82.7	77.0	69.3	60.4	52.4	46.9	43.3	37.6
F <sub>a</sub>	1.69	2.65	1.97	1.57	1.40	1.24	1.09	1.21
Regression <sup>b</sup>	+ 0.24	+ 0.17	+ 0.03	- 0.02	- 0.10	- 0.18	- 0.27	- 0.38
Jersey, n = 163								
V, between groups	125.9	54.9	79.5	83.6	93.1	93.8	93.9	94.3
V, within groups	74.5	56.0	46.8	45.3	41.7	38.3	36.5	35.3
F <sub>a</sub>	1.69	1.02	1.70	1.85	2.23*	2.45*	2.57*	2.67*
Regression <sup>b</sup>	- 0.56	- 0.48	- 0.71	- 0.65	- 0.74	- 0.78	- 0.84	- 1.01

<sup>a</sup> Test of significance, \* Significant at the 5 per cent level.

<sup>b</sup> Change in 1000 FCM/W for an increase of 100 in W.

2-month, and 3-month partial lactations the regression is positive, that is, the large cows yield more milk energy per unit initial live weight than the small cows. The regression changes progressively as the length of partial lactation increases.

In the case of the Jersey records, the regressions are negative throughout and show a progressive change similar to that for the Holsteins. For partial lactations of 1 to 4 months, the difference between live weight groups is below the 5 per cent level of significance; for partial lactations of 5 to 8 months, the difference is above the 5 per cent level but below the 1 per cent level of significance.

The evidence of table 4 is that  $FCM/W$  is substantially independent of  $W$  for short periods of lactation but a negative correlation tends to appear as the length of period used increases.

Dealing with all the records, table 5 presents an analysis of covariance designed to show the relation between  $W$  and  $FCM/W$ , in total, and segregated as between herds and within herds. In the Holstein records the total correlation starts as +.03 in the 1-month records and finishes as -.12 in the 8-month records. While the latter is significant at the 1 per cent level, the actual decrease in 1000  $FCM/W$  is not large, amounting to only .4 for an increase of 100 in  $W$ . In the Jersey records the total correlation between  $W$  and  $FCM/W$  is below the 5 per cent level of significance throughout. These total correlations suggest the conclusion that  $FCM/W$  is independent of  $W$ .

When the correlation between  $W$  and  $FCM/W$  is considered as between herds and within herds, there is throughout a relatively large positive correlation as between herds and a relatively small negative correlation within herds. That is, large-cow herds yield more milk energy per unit size of cow than do small-cow herds. But within a herd, there is a tendency for a large cow to yield less milk energy per unit size than her small herd mates.<sup>3</sup>

<sup>3</sup> The negative regression of  $FCM/W$  on  $W$  within herds is of an order of magnitude that might be expected on the theory that milk-energy yield is proportional to the  $3/4$  power of  $W$ . This suggests the interpretation that real size, "metabolic body size" or "physiologic weight," is proportional to the  $3/4$  power of  $W$  rather than to  $W$  itself. The positive and substantial regression of  $FCM/W$  on  $W$  as between herds would be explained as due to differences in herd management with a pronounced tendency for herds made up of large cows to have more favorable conditions than herds made up of small cows. An alternative to the  $3/4$  power interpretation is the interpretation (here preferred) that the milking potentialities of the large cows within a herd are not as fully realized as in the case of the smaller herd mates.

The fact that the relation between  $W$  and  $FCM/W$  is different between herds than it is within herds raises a question in connection with figure 6. Is the relative influence of weight and age on milk-energy yield the same within herds and between herds as it is in total as pictured in figure 6? The present records are not adequate to deal with this question.

TABLE 5  
*Analysis of variance and covariance with respect to W and FCM/W, records classified by herds (30 Holstein herds, mean W, 986 to 1395; 21 Jersey herds, mean W, 745 to 955)*

1000 FCM/W for partial lactation of						
	1 mo.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.
Holstein, n = 957						
V, between herds	362	348	322	295	274	251
V, within herds	56	47	41	37	34	30
F <sub>8</sub>	6.46**	7.40**	7.85**	7.97**	8.06**	8.37**
Correlation <sup>b</sup>						
Total	+ 0.03	- 0.01	- 0.02	- 0.04	- 0.05	- 0.08*
Between herds	+ 0.33	+ 0.28	+ 0.23	+ 0.23	+ 0.23	+ 0.23
Within herds	- 0.03	- 0.07*	- 0.08*	- 0.10**	- 0.12**	- 0.17**
Regression <sup>c</sup>						
Total	+ 0.13	- 0.03	- 0.09	- 0.15	- 0.21	- 0.28
Between herds	+ 1.60	+ 1.31	+ 1.06	+ 1.02	+ 0.96	+ 0.98
Within herds	- 0.17	- 0.30	- 0.31	- 0.38	- 0.44	- 0.53
Jersey, n = 195						
V, between herds	210	187	164	157	138	129
V, within herds	64	47	39	33	31	29
F <sub>8</sub>	3.28**	3.98**	4.21**	4.56**	4.76**	4.45**
Correlation <sup>b</sup>						
Total	- 0.02	- 0.01	- 0.03	- 0.02	- 0.03	- 0.04
Between herds	+ 0.51*	+ 0.50*	+ 0.48*	+ 0.47*	+ 0.45*	+ 0.45*
Within herds	- 0.18*	- 0.18*	- 0.29*	- 0.19*	- 0.21**	- 0.22**
Regression <sup>c</sup>						
Total	- 0.13	- 0.04	- 0.16	- 0.10	- 0.18	- 0.22
Between herds	+ 4.32	+ 4.00	+ 3.57	+ 3.47	+ 3.25	+ 3.08
Within herds	- 1.25	- 1.06	- 1.10	- 1.00	- 1.05	- 1.05

\* Test of significance, \* Significant at the 5 per cent level, \*\* Significant at the 1 per cent level.

<sup>b</sup> Correlation between W and FCM/W.

<sup>c</sup> Change in 1000 FCM/W for an increase of 100 in W.

## FATNESS AT CALVING AND FCM/W

At the time of determining live weight of the individual cows in the present trial the tester also made a score of the cow intended to describe her condition of flesh. The scheme of scoring was based on a scale from 1 to 9, in which an excessively thin condition was scored as 1 and an excessively fat condition was scored as 9, all on the somewhat indefinite verbal expressions: thin minus = 1, thin = 2, thin plus = 3, medium minus = 4, medium = 5, medium plus = 6, fat minus = 7, fat = 8, fat plus = 9. Scores were made as above for 761 of the 957 records of Holstein cows, and 183 of the 195 records of Jersey cows. The results are given in table 6. The differences between

TABLE 6  
*Analysis of variance, records classified by score of cow with respect to fatness at calving (see text)*

	1000 FCM/W for partial lactation of							
	1 mo.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.	7 mos.	8 mos.
<b>Holstein, n = 761</b>								
V, between groups	89.8	72.3	66.2	51.6	49.3	34.4	35.2	37.5
V, within groups	63.8	55.0	49.0	44.0	41.5	36.7	33.9	31.8
F <sup>a</sup>	1.41	1.31	1.35	1.17	1.19	1.07	1.04	1.18
Regression <sup>b</sup>	+ 0.66	+ 0.51	+ 0.46	+ 0.36	+ 0.31	+ 0.15	+ 0.12	+ 0.07
<b>Jersey, n = 183</b>								
V, between groups	78.1	31.1	25.0	27.0	25.9	27.2	27.5	36.1
V, within groups	79.4	61.4	52.2	49.1	45.5	41.5	39.2	37.1
F <sup>a</sup>	1.02	1.97	2.09	1.82	1.76	1.53	1.43	1.02
Regression <sup>b</sup>	+ 1.03	+ 0.55	+ 0.98	- 0.12	- 0.36	- 0.57	- 0.64	- 0.83

<sup>a</sup> Test of significance, all are below the 5 per cent level.

<sup>b</sup> Change in 1000 FCM/W for an increase of 1 in fatness score.

groups on the basis of fatness at calving are well below the 5 per cent level of significance in all cases. Hence it appears that FCM/W is independent of fatness of the cow at calving.

## DISCUSSION

The results of the present investigation bear directly on the postulates previously advanced (4) that FCM/W for the 8-month partial lactation is independent of:

1. Service period and length of lactation.
2. Age of cow at start of lactation.
3. Fatness of cow at start of lactation.
4. Live weight of cow at start of lactation.
5. Composition of milk for the lactation.
6. Breed of cow.

Of these postulates 1 is automatic and 5 has been heretofore amply demonstrated for various other records. Postulates 2 and 3 are good on the evidence of the present paper. Postulate 4 is good within either the Holstein or Jersey breed on the present evidence. Postulate 6 is still a question.

In the present records the Jersey breed is far above the Holstein breed on the basis of FCM/W. This is interpreted to mean that the present Jersey cows are superior to the Holstein cows, either in point of inherent proclivity to lactation (FCM/W), or in point of management, or both (probably both). There can hardly be any question as to the superiority of the Jersey records since the 822-pound average Jersey of table 1 produced in the 8-month partial lactation an absolute 10,030 Calories of milk energy per day (estimated as  $29.5 \times 340$ ) while the 1211-pound average Holstein of table 1 produced an absolute 9,928 Calories per day (estimated as  $29.2 \times 340$ ). That is, the small Jersey did more work in lactation than did the large Holstein.

It is clear that the performance of the Jersey cows excels that of the Holstein cows in the present data; whether it excels to the extent indicated by FCM/W may be open to question. The dairy cow is undoubtedly undergoing evolution under the pressure of artificial demands and selection. Can that evolution be pressed to the point of equality with respect to FCM/W as between large and small breeds of cows? Apparently, it has already reached that point within the large breed, or within the small breed, (cf. 1, footnote 11).

As between the use of FCM/W or the use of age-corrected FCM the case is entirely clear. Age is often unknown, while live weight is always determinable in a system of record keeping. Furthermore, the physiological basis of increase in yield with age appears to be associated with increase in size of the cow with age, age of itself being a practically negligible factor. The use of age correction is so widespread and deeply rooted that any question of its soundness may meet with opposition, and a demand for more extensive investigation before acceptance. It should be held in mind that the argument here presented is based on initial live weight\* (within the first 30 days after calving, preferably about the 3rd-5th day) and on milk-energy yield for the first 8 months of the lactation.

#### SUMMARY AND CONCLUSIONS

The data used consist of 957 records of Holstein cows and 195 records of Jersey cows for partial lactations of 1, 2, 3, 4, 5, 6, 7, and 8 months in Illinois Dairy Herd Improvement Associations. The records are computed to a daily milk-energy basis in terms of 4 per cent milk in pounds (FCM). Live weight in pounds (W) within the first 30 days after calving is a part of the record. Age is known for 255 Holstein records and 163 Jersey records.

\* Where live weight is estimated after the close of the record period, as for example, in the extensive early 12-months Register of Merit records of the Jersey breed, an entirely different relation appears between age, weight and yield, as compared with the relation found in the present work. Final live weight and initial live weight may be quite different and have different biological significance. It is here considered essential to deal with initial live weight.

The data are studied by graphic portrayal, by fitting equations and by analysis of variance.

By fitting the equation,  $FCM = a + bW + dA$ , where  $A$  = age, it is shown that the influence of age on FCM independent of weight is entirely negligible in the Jersey records and practically so in the Holstein records (see figure 6). By fitting the equation,  $FCM = BW + DA$  it is shown that  $D$  is practically zero in the Jersey records and very small in the Holstein records. Hence,  $FCM = BW$  or  $FCM/W = B$ , a constant so far as age is concerned, and  $FCM/W$  should be independent of age. Analysis of variance applied to the records classified by age shows that differences between age groups (2 to 13 years, by years) are in fact well below the 5 per cent level of significance.

It is concluded that the use of age correction factors is justified only in case  $W$  is unknown and then only insofar as  $W$  is associated with age and correctly portrayed by the factors used.  $FCM/W$  may be used freely without regard to age of cow. As rapidly as feasible initial live weight at each lactation should be made a part of all dairy records and  $FCM/W$  (or similar principle) should supersede the biologically unsound principle of age correction.

Analysis of variance and covariance applied to the records classified by  $W$  shows that differences between the live weight groups with respect to  $FCM/W$  are below the 5 per cent level of significance for the shorter partial lactations but become increasingly significant up to the 8-month partial lactation. In general, the regression of  $FCM/W$  on  $W$  is negative and in the gross amounts to a decrease of about .4 in 1000  $FCM/W$  for an increase of 100 in  $W$ . However, as between herds an opposite tendency prevails and as between Jersey herds, there is an increase of 2.73 in 1000  $FCM/W$  for an increase of 100 in  $W$ .

It is concluded that as between cows of different live weights in either the Holstein or Jersey breed,  $FCM/W$  affords an equitable criterion of dairy merit or development.

Analysis of variance applied to records classified by fatness of cow at calving shows no significant differences between groups, and it is concluded  $FCM/W$  is independent of fatness of cow at calving.

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## THE FAT METABOLISM OF THE MAMMARY GLAND<sup>1</sup>

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Certain aspects of the fat metabolism of the mammary gland have been given considerable attention for a number of years. In 1912, Foa (6) using the perfusion technique concluded that milk fat was formed from neutral fat. When olive oil was added to the perfusion solution, the fat in the fluid obtained from the gland had a lower iodine number than that of the olive oil. The work of Meigs, Blatherwick and Cary (17) was accepted as proof that milk fat was formed from blood phospholipids until Blackwood and Sterling (2) showed that this work was invalidated by a difference in the concentration between the jugular and the mammary venous bloods. Lintzel (15) working with goats in which arterial blood was obtained by heart puncture demonstrated the loss of neutral fat to the mammary gland. Graham, Jones and Kay (9) in a similar study with cows in which the arterial blood was obtained from the internal iliac by rectal puncture concluded that, in the main, milk fat is derived from the non-phosphatide fatty acids of the blood. The use of neutral fat by the active gland was also demonstrated by Maynard *et al.* (16) in a series of experiments in which the arterial blood was obtained from the internal pudic artery through the vaginal wall. Later, Graham *et al.* (8) reported that the respiratory quotient of the lactating gland of the goat exceeded unity suggesting that fat was possibly being synthesized from some carbohydrate material. A study of the relative amount of blood fat used by the mammary gland of the cow has been in progress in the Minnesota laboratory for some time and some preliminary notes have been published, Shaw and Petersen (20, 21).

Using the Evelyn-Salter (5) method for the determination of hemoglobin, Shaw and Petersen (24) found that with any excitation or disturbance of the animal there were invariably large blood volume changes in the mammary gland regardless of the rapidity with which the samples were taken. These changes did not occur in the mammary gland of the com-

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pletely undisturbed animal. Corrections of arteriovenous differences on the basis of blood volume changes produced values which were obviously untenable.

These findings not only account for many of the unexplained variations in arteriovenous differences found in the data of all workers, but serve as a very valuable tool in determining whether or not the arteriovenous differences can be expected to represent the normal metabolism of the gland.

In a previous communication, Shaw and Petersen (21) reported that the quantity of blood fat used by the mammary gland increased with the increase in time following milking. This paper deals with the explanation of this and other phenomena associated with the fat metabolism of the mammary gland.

#### EXPERIMENTAL

For the arteriovenous studies the venous blood was drawn from the subcutaneous abdominal vein. In most of the experiments, the skin was anesthetized at the point of venipuncture with ethyl chloride since any disturbance to the animal is usually due to the venous and not the arterial puncture. The arterial blood was obtained by rectal puncture from either the prepubic or internal iliac arteries. The arteriovenous data unless otherwise reported includes only those experiments in which there were no detectable blood volume changes and in which the animals showed no sign of disturbance.

The following chemical techniques were used: hemoglobin, Evelyn-Salter (5); blood fat, Allen (1); blood glucose, Shaffer and Somogyi (19); plasma calcium, Clark-Collip (3); and, plasma phosphorus, Fiske and Subbarow (7). The obstetrical pituitrin used, being predominately oxytocin, will be referred to as such.

More than 200 arteriovenous blood fat differences have been determined since the study of the amount of blood fat used by the lactating gland was initiated in 1936. However, we were unable to obtain an orderly picture until the relationship of excitation to blood volume changes in the gland was established. Practically all of the data reported in this communication were obtained from the Holstein herd at the University of Minnesota.

In 52 of the analyses of blood fat differences, there were no measurable blood volume changes in the gland. This data is presented in figure 1. As will be observed, there is little or no blood fat lost to the gland immediately after milking. In two cases there was actually a passage of fat back into the blood. Following the period immediately after milking, there was a slowly increasing uptake of blood fat by the gland for a period of about four hours after which the fat was used at a more constant rate, although the three highest values are to be found nine and ten hours after milking. In two cases, the cows were not milked out for a period of more than 15 hours. The

results of blood samples taken at this time show that the passage of fat into the gland had almost ceased. The failure of the gland to use fat at this particular time is undoubtedly due to the pressure built up in the gland.

The passage of blood calcium into the lactating gland presented a similar picture. It will be seen from figure 2 that milking retarded the uptake of calcium considerably. The building up of considerable pressure in the gland at the end of 15 hours also almost completely stopped the use of blood calcium. However, blood glucose continued to be used by the gland at this time in normal amounts, the arteriovenous change being 14.6 and 11.4 mg. per cent in the two experiments cited.

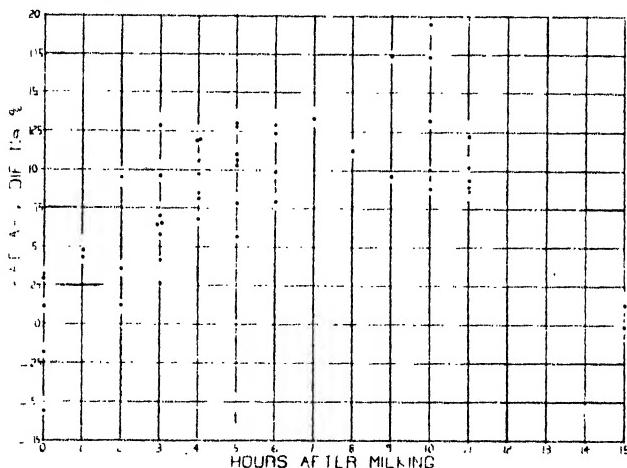


FIG. 1. Arteriovenous differences in blood fat in relation to time after milking.

The use of blood calcium by the gland is considered as the best indirect measure of the volume of blood passing through the gland per unit volume of milk. The more direct measures have not been considered because of the accompanying excitation and because the analyses are necessarily too limited to give any adequate picture of the loss of the various substances to the mammary gland. From the great variations in individual animals in arteriovenous differences and in the rate of blood flow as shown by the uptake of oxygen (18), it is apparent that balances on the mammary gland can not be considered significant unless large numbers of data are available from experiments in which blood volume changes have not occurred. The errors involved become minimized as the number of good observations increase. Calcium, therefore, was used in an attempt to determine what per cent of the milk fat was derived from blood fat. The average loss of fat to the gland in the data presented in figure 1 was 9.0 mg. per cent. The average calcium loss to the gland in the data presented in figure 2 was 0.29 mg. per cent. The values obtained 15 hours after milking were not in-

cluded because the glands were abnormally distended at that time. On the basis of 120 mg. per cent of calcium in the milk approximately 410 volumes of blood plasma would be required to provide milk calcium. Similarly, on the basis of 3500 mg. per cent of fat in the milk, the volume of blood plasma required to produce the milk fat is approximately 390. Even allowing for considerable error in these calculations, it is apparent that most of the milk fat is derived from the blood fat. Recently some observations have been made which further favor the conclusion that most of the milk fat is derived from blood fat. In a series of operations on the glands of cows and goats, it was observed that the flow of lymph was very large and indicated that any attempt to conduct a balance of the mammary gland would have to include lymph. An analysis of the lymph demonstrated that while considerable calcium was removed from the gland by the lymph, very little fat was carried away in this manner. These data will be presented elsewhere in a later communication.

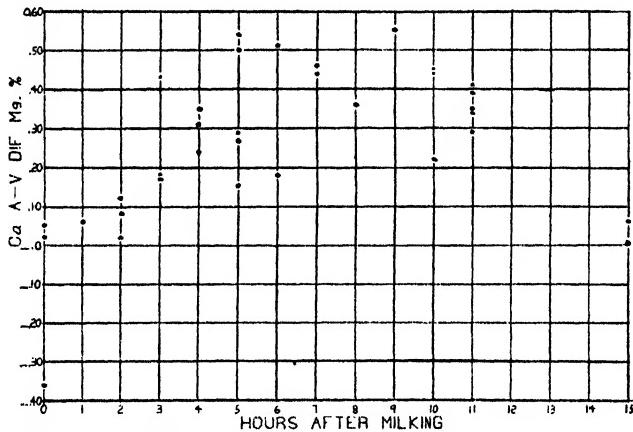


FIG. 2. Arteriovenous differences in blood calcium in relation to time after milking.

In the method used for fat determination neither phospholipids nor free fatty acids are recovered. The relatively large amount of fat shown to be used by the gland by this method is therefore limited to neutral fat, cholesterol and cholesterol ester fractions.

Several experiments were conducted to further study the peculiar effect of milking upon the uptake of blood substances by the lactating gland. One half of the udder was milked out, leaving the other half distended with milk. Arterial blood samples and right and left venous samples were then taken simultaneously. The results of a series of such experiments are presented in table 1. Unfortunately, in several of these experiments, hemoglobin determinations were not made. The data are presented, however, because the striking effect noted in these cases occurs consistently. Only a small quantity of blood fat was taken up by the gland on the side which had

TABLE 1

*The effect of milking out one half of the udder upon the use of blood substances by both right and left glands*

	No. of observations	Arterial	Left venous	Right venous (side milked out)
Blood Fat mg. %	1*	239.7	235.6	238.5
	2	233.5	219.5	229.7
	3	201.9	187.1	195.6
	4	162.2	153.3	158.6
	5	214.2	200.0	209.9
	6	199.5	188.9	198.3
	7*	182.7	173.4	179.2
Calcium mg. %	8*	10.00	9.32	9.95
	9	8.20	7.98	8.20
	10	9.66	9.49	9.60
Phosphorus mg. %	11*		4.92	5.47
Amino-Acids mg. %	12	4.41	4.00	4.00
	13	3.90	3.62	3.58
Glucose mg. %	14	64.0	55.6	56.0
	15	64.8	58.2	58.0
	16	74.0	56.6	56.6
	17*	50.0	44.6	43.4

\* No blood volume changes.

been milked out. Blood fat continued to pass into the unmilked side rather freely, however. It, thus, became apparent that the presence of milk in the gland was necessary to facilitate the normal transfer of fat from the blood to the secretory tissue of the gland. The same appeared to be true of both calcium and acid soluble phosphorus. In observation number 11, no arterial sample was drawn. That the milked side, however, used less phosphorus than the unmilked side is shown by the fact that in the former case the level of venous blood phosphorus was higher, indicating that less phosphorus had been removed. Both sides continued to use glucose and amino acids in equal amounts which was to be expected from previous findings in which it was shown that the arteriovenous differences of glucose and amino acids were not affected materially by the time interval after milking, Shaw, Boyd and Petersen (22), Shaw and Petersen (23).

These results were quite surprising inasmuch as it was thought that if the milking out had any effect it would be to increase the arteriovenous differences. With the release of the pressure in the gland by the removal of the milk, it might be expected that there would be an increased passage of certain substances into the gland. Such however, is not the case. The determination of hemoglobin shows that it is not merely a matter of concentration of the blood at this time.

Another series of experiments dealing with the effect of oxytocin upon arteriovenous differences serves to still further explain many of these phenomena. Since the work of Ely and Petersen (4) had demonstrated that oxytocin or an oxytocic-like principle must be responsible for the ejection of

milk, it was suspected that the failure of the gland to remove fat and other substances from the blood following milking was associated with the effect of this principle upon the gland.

Accordingly, the effect of injections of oxytocin upon the use of blood fat by the mammary gland was studied. A number of cows were injected with 10 I.U. of oxytocin after arteriovenous blood samples had been drawn, following which arteriovenous blood samples were again drawn. Usually the oxytocin was injected into the mammary vein into which a hypodermic needle had already been placed in obtaining the initial venous blood sample. In table 2, four such experiments are recorded in which no significant blood volume changes occurred. Samples were drawn from Cow Number 612 im-

TABLE 2

*The effect of injections of oxytocin upon the use of blood fat by the mammary gland*

		Hemoglobin %	Plasma fat mg. %	Remarks
Cow No. 612 Immediately after milking	Arterial	12.18	298.7	Before injection of oxytocin
	Venous	12.18	300.9	
	Arterial	12.18	294.8	After injection of oxytocin
	Venous	12.18	299.9	
Cow No. 615 2 hours after milking	Arterial	12.18	277.0	Before injection of oxytocin
	Venous	12.18	270.6	
	Arterial	11.87	277.2	After injection of oxytocin
	Venous	11.87	276.5	
Cow No. 449 10 hours after milking	Arterial	13.29	306.2	Before injection of oxytocin
	Venous	13.29	288.9	
	Arterial	13.29	301.3	After injection of oxytocin
	Venous	13.29	298.6	
Cow No. 577 Right side milked out	Left venous	12.95	184.4	Before injection of oxytocin
	Right venous	12.95	195.8	
	Arterial	12.92	192.5	After injection of oxytocin
	Left venous	12.97	191.2	
	Right venous	12.95	190.9	

mediately after milking. An increase of 2.2 mg. per cent in venous blood fat was observed. Following the injection of oxytocin, there was an increase in the venous blood fat of 5.1 mg. per cent. This procedure was repeated with Cow Number 615 two hours after milking. The arteriovenous fat difference of 7.6 mg. per cent decreased to 0.7 mg. per cent after the injection of oxytocin. The procedure was then repeated with Cow Number 449 ten hours after milking. The fat loss of 17.3 mg. per cent was decreased to 2.7 mg. per cent by the injection of oxytocin.

In the experiment with Cow Number 577, the right half of the gland was milked out and venous samples were taken simultaneously from both the

right and left mammary veins. With the needles remaining in the veins, oxytocin was injected and both right and left venous blood samples were drawn simultaneously with an arterial sample. Since the level of venous blood fat prior to the injection of oxytocin was higher on the side milked out than on the unmilked side, it was apparent that the unmilked side was using more blood fat. Following the injection of oxytocin, neither side used any appreciable quantities of blood fat. From the results of these experiments, it was apparent that intravenous injections of oxytocin almost completely inhibited the uptake of blood fat by the lactating mammary gland even in glands filled with milk. This was unexpected since it had previously been shown that the inhibiting effect of milking upon the use of blood fat by the gland did not occur until after the milk had been removed. It is believed, however, that the two effects are both due to oxytocin, and that the effect of injections of oxytocin upon the unmilked gland are due to the use of this principle in excess of physiological dosage.

In the experiments with the undisturbed animal in which there were no blood volume changes in the gland, the use of blood calcium and blood phosphorus appears to follow that of the fat. During excitation, however, the calcium and phosphorus presents an extremely varied picture and the arteriovenous differences are unpredictable. This is not true of blood fat. In an earlier communication, it was demonstrated that with a concentration of the blood in the gland excessive amounts of fat passed into the gland while with a dilution of the blood in the gland the fat often passed back into the venous blood. In a number of experiments, observations were made of the effect of oxytocin upon the arteriovenous fat differences in the gland of the excited cow. It will be observed in a typical experiment reported below with Cow Number 446 that the expected variations did not occur.

<i>Cow 446</i>	<i>Hemoglobin</i>	<i>Plasma fat</i>	
	%	mg. %	
Arterial	14.25	255.0	After injection of oxytocin.
Venous	14.50	259.7	Cow excited.

With the concentration of the blood of 1.1 per cent, it was expected from previous experience that considerable blood fat would be retained in the gland. On the contrary, however, due to the effect of oxytocin, blood fat was concentrated in the venous blood to approximately the same extent as hemoglobin, indicating that here were no interchanges of fat between the gland and the blood plasma at this time. The injection of oxytocin also hindered the passage of calcium and phosphorus into the gland of the excited cow from time to time, but the effect was not as marked as in the case of fat.

#### DISCUSSION OF RESULTS

Following milking very little blood fat was taken up by the gland and, in some cases, immediately after milking, there was actually a passage of fat back into the venous blood. With the increase of the time interval following milking, blood fat was used in increasing amounts until about four hours after milking, after which there was a more constant uptake of fat by the gland. In some cases, the amount of fat used by the gland continued to increase for several hours longer.

The decreased use of blood fat by the lactating gland following milking was found to be associated with the removal of the milk from the gland. The stimulus of the "letting down" of milk did not in itself prevent the passage of blood fat into the gland. Injections of oxytocin, in apparently greater than physiological amounts, usually completely prevented the uptake of blood fat by the gland regardless of the time interval after milking.

Blood calcium and blood phosphorus were influenced in the same direction but with any excitation or changes in blood volume in the gland the results were more unpredictable. The uptake of glucose and amino acids, however, did not materially change.

In dealing with the transfer of substances from the blood to the gland, and from the gland to the blood, it appears that two phases must be reckoned with. Consideration must be given not only to the equilibrium between the blood plasma and the tissue fluid, but also to the equilibrium existing between the tissue fluid and the secretory cells of the gland. It can be assumed, as suggested by Starling, that the blood pressure decreases progressively along the capillary, being greatest at the arterial and least at the venous end. At the arterial end of the capillary, the hydrostatic pressure exceeds the colloidal osmotic force and fluid is forced from the capillary. At the venous end, the colloidal osmotic force exceeds the blood pressure and fluid passes from the tissue spaces into the capillary. The capillaries in the mammary gland of the lactating cow are apparently quite permeable to plasma fat as well as water, salts and a certain amounts of plasma proteins. It is believed that there is a continuous and relatively large flow of fluid between the blood plasma and the tissue spaces of the gland containing considerable plasma fat and other substances. A temporary change in blood pressure, pressure within the gland, or changes in the permeability of the capillaries would tend to alter, at least momentarily, the normal equilibrium existing between the plasma and the tissue fluid. These changes undoubtedly account at least in part for the variations in the arteriovenous differences in excited cows reported in an earlier communication (24). The mechanism by which oxytocin causes fat and calcium to increase in the venous blood after the gland has been milked out may be explained as being due to a greater momentary pressure in the tissue spaces following the constriction of the smooth muscle of the gland. This would

tend to offset the blood pressure in the venous end of the capillary more completely and possibly even exceed the force of the blood pressure at the arterial end of the capillary.

The effect of injections of oxytocin in preventing the passage of certain blood substances into the secretory tissue of the gland may be explained in part on the same basis. Indeed, it may well be that the complete contraction of the smooth muscle can not occur until the milk has been removed from the alveoli. However, it has been shown by Hammond (10) and others that the pressure is greatest following stimulation of the letting down of milk when the gland is still filled with milk. More significant, however, is the fact that fat is not used in normal amounts until three to four hours after milking and in some cases the increase in the use of fat continues for several more hours. It is highly improbable that the contraction of the smooth muscle of the gland by oxytocin would continue this length of time.

It is believed that during the process of milking the alveoli collapse rather completely due to oxytocin which results in a decrease in the permeability of the basement membrane of the secretory cells to blood fat and possibly other substances. With the gradual engorgement of the cells and the filling of the alveoli with milk, the basement membrane becomes increasingly permeable to the lipides in the fluid which is circulating between the plasma and the tissue spaces. The more freely diffusible substances, such as glucose and amino acids, are apparently not materially affected by these changes. The passage of substances from the interstitial fluid to the cells must be governed not only by the permeability of the basement membrane but also by the rate at which they are used for synthesis in the secretory cells. The fact that calcium and phosphorus are affected in a similar manner to that of fat may mean that they are taken in, in part, by combination with other materials such as calcium proteinate and in connection with fat phosphorylation in the transport of fat across the basement membrane of the secretory cells of the gland.

The work of Kelly (13) and Kelly and Petersen (14) has shown that there is considerable lipase in the lactating gland and that only free fatty acids are present in the basal part of the cell. This suggests the possibility that the action of lipase may be necessary in the transport of fat across the basement membrane. However, it is possible that the fat is not acted upon by lipase until it passes into the cell and that the action of lipase is so rapid in the basal portion of the cell that the fat is hydrolyzed before the material can be fixed and stained and therefore could not be detected in the cells of the excised gland.

The effect of oxytocin in preventing the loss of fat to the milk-filled gland is probably due to a partial collapse of the alveoli and the building up of excessive pressures in the gland which occurs following the injection of oxytocin.

The increasing uptake of blood fat by the gland with the increase in the time interval after milking would be explained by the gradual distension of the alveoli and the engorgement of the cells which makes the basement membrane increasingly permeable to the fat.

It is apparent from the observations herein reported that most of the milk fat is derived from the blood fat. These results are not in agreement with the suggestion of Smith and Dastur (25) that oleic acid is synthesized from carbohydrate and that the short chain fatty acids are by products of this synthesis. They found that during inanition there was a decrease of about 80 per cent in the original content of the lower fat acids, a deficiency which was almost entirely made up by an increase in oleic acid. If oleic acid were synthesized from carbohydrate material, it would be extremely difficult to explain why the gland used such large quantities of blood fat since oleic acid makes up about 32 per cent of the total milk fat.

On the basis of the quantity of fat used by the gland, it appears more likely that this apparent relationship between oleic acid and the short chain acids is due to a breakdown of oleic glycerides as suggested by Hilditch and Thompson (12), Hilditch and Paul (11) and Shaw and Petersen (20). If this is true, then some other explanation must be found for the high respiratory quotient reported by Graham *et al.* The data of Lintzel is of little help in this regard since his blood samples were drawn shortly after milking at which time the arteriovenous fat differences would be expected to be small. In fact, if the milk fat of the cow and the goat is produced from similar blood precursors, the arteriovenous fat differences in goats should be about twice that found in cows because the volume of blood per unit volume of milk as reported by us here and elsewhere is about double that reported for goats.

#### CONCLUSIONS

1. Very little blood fat is taken up by the gland immediately after milking. With the increase of the time interval following milking, blood fat is used in increasing amounts until about four hours after milking, after which time the fat is used in more constant amounts. Calcium appears to present a similar picture. The absorption of fat and calcium ceases about 15 hours after milking. Glucose continues to be used in normal amounts, however.
2. The passage of blood fat into the gland can be prevented by intravenous injections of oxytocin.
3. The passage of blood fat into the lactating gland and to a lesser extent of calcium and acid soluble phosphorus is associated with the distension of the alveoli and the filling of the secretory cells with milk. Blood glucose and amino acids are not similarly affected but continue to be used in fairly constant amounts. A hypothesis is developed on the basis of the observations to account for the passage of blood substances into the glandular tissue.
4. The quantity of blood fat used by the gland is sufficient to account

for all of the milk fat and justifies the conclusion that but little milk fat is produced from other substances.

5. The average use by the lactating gland of the cow of 9.0 mg. per cent of plasma fat is limited to neutral fat and/or cholesterol fractions.

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## THE EFFECT OF STORAGE TEMPERATURES UPON CERTAIN CHARACTERISTICS OF BOVINE SEMEN

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Motility of spermatozoa has been widely studied and at one time motility was identified with fertilizing capacity. While this determination was easily made, some workers, (3, 12, 13) believed that motility was not necessarily a criterion of fecundity of spermatozoa. Williams and Savage (15, 16) in their investigations of bulls, showed that no great dependability could be placed upon the motility of spermatozoa. Lagerlöf (11) found that in sterile bulls and in those of reduced fertility, there was great variability in the motility of spermatozoa. Donham *et al.* (8) found a definite correlation between the conception rate of cows and motility of the spermatozoa, and they stated that semen which contained less than 90 per cent of active spermatozoa should be regarded as abnormal, since it did not insure satisfactory fertilization. While a high percentage of active motility of spermatozoa does not guarantee the fecundity of the semen, it is, therefore, likely, according to the workers cited, that reduced motility would indicate reduced fertility or even sterility.

Various other criteria have been set up for the evaluation of the fecundity of bovine semen specimens. Volume, concentration of spermatozoa, and abnormalities of spermatozoa have been suggested as important factors related to fecundity of semen. The standards formulated were the result of studies of semen from bulls with disturbed fertility (1, 2, 15, 16). An analysis of semen samples of a group of fertile bulls would seem to furnish a better basis of appraisal of fertilizing capacities of sperm cells. In a previous study of fresh semen samples obtained from 11 fertile bulls (6), it was shown that there were certain relationships between volume of semen sample, percentage of progressive motility, concentration of spermatozoa, and pH value of the semen. The fertilizing capacity of the semen appeared to be dependent upon a combination of these factors rather than a single one. It might be mentioned that the mean number of spermatozoa per mm.<sup>3</sup> in the samples from the 11 fertile bulls was 734,000 with a range of from 8,000 to 1,997,000 in a total of 266 ejaculates in studies of the first, second, and third successive ejaculates (6). The mean number of the 168 samples

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of the first ejaculate was 826,000. Anderson (1, 2) concurrently reported a mean concentration of 873,000 per mm.<sup>3</sup> with a range of from 510,000 to 1,875,000 in samples obtained from six fertile bulls. The number of ejaculates studied was not mentioned nor which ejaculate. Anderson showed also that the percentage of abnormal spermatozoa in fertile bulls was 8.1; in bulls of reduced fertility 13.1; and in sterile bulls 17.6 per cent. Unpublished data at the University of Nebraska dairy herd have revealed that atypical spermatozoa are of normal occurrence in the case of fertile bulls. Hotchkiss *et al.* (10) in a detailed analysis of 200 fertile men whose wives were in the first half of gestation arrived at similar conclusions regarding characteristics and fecundity of semen samples. They observed further that atypical spermatozoa were not so great a factor affecting fecundity as had been concluded by some investigators since these abnormal cells were found in the semen of the 200 fertile men. From these observations it may be concluded that abnormal cell count, at least when the proportion is relatively small, is of minor importance in evaluating the fecundity of a semen sample.

In this study the influence of four temperatures during storage upon the motility and the pH values of semen samples obtained from 11 fertile bulls and grouped according to the initial percentage of progressive motility of spermatozoa, concentration of spermatozoa per mm.<sup>3</sup> and pH, is presented. The characteristics of the semen of the bulls from which the samples were obtained are presented and conceptions resulting from insemination with both fresh and stored semen are listed.

#### PROCEDURE AND METHODS

The semen samples were collected from 11 fertile bulls of the Jersey, Guernsey, Ayrshire, and Holstein breeds over a period of approximately nine months. The bulls ranged in age from one year and two months to eight years (table 4). All were free from contagious diseases. For housing, the bulls were kept in a semi-closed shed and each was allowed to run in a paddock for a half day, each day. A limited quantity of alfalfa hay was fed daily and grain was fed at the rate of between three-fourths of a pound and one pound for each 100 pounds of live weight. The grain mixture contained about 14 per cent of digestible protein and one per cent each of steamed bone meal and iodized salt.

The regular procedure was to take a semen sample every third day but sometimes only one sample was taken during a week. Semen samples were taken with the Cambridge type artificial vagina (14) and the ejaculating bull was allowed to mount either a protected cow or another bull. Each semen sample collected was kept separate and the second and third ejaculates were taken immediately after the first. Care was taken to wash the prepuce and irrigate the sheath of the bull with warm water before the first

semen sample was taken. Aseptic precautions were followed carefully in the collection, handling, and storing of semen samples. All equipment coming in contact with semen was first rinsed with diluter. The diluter used was made according to a Russian formula (14) by dissolving 13.6 grams of sodium sulfate, 12 grams of anhydrous glucose, and 5.0 grams of salt-free peptone in one liter of distilled water and sterilizing at a steam pressure of 13 pounds for 30 minutes in an autoclave. No diluter was added to any sample of semen. Each ejaculate was emptied, as soon as possible after collection, into a sterile cotton-stoppered test tube and this in turn was placed in a larger test tube where it rested on a cork at the bottom. The double test tube was then placed in a pail of water at a temperature between 50° and 60° F. except for samples to be stored at 70° F. This method cooled the semen sample at the rate of about one degree F. per minute and cooling was completed in thermostatically controlled storage boxes.

A microscope fitted with a low-power objective and equipped with a slide and coverslip preparation in a stage incubator at a temperature of 102° F. was used to determine the motility which was estimated to the nearest 10 per cent of progressive motion of spermatozoa across the field. Initial motility was determined within an hour after the semen sample was taken. The determination of pH values was made at a temperature of 77° F. (25° C.) with a potentiometer equipped with a quinhydrone gold electrode. The concentration of spermatozoa per min.<sup>3</sup> was determined by the use of a haemocytometer using standard procedure. The ejaculates of semen varied in volume and each ejaculate was divided into one-cc. samples which were stored at various temperatures until observed. The storage chambers were equipped with thermostatic switches and maintained temperatures with a variation of one degree F. The observations recorded were made only on semen samples which showed spermatozoa in progressive motion. The procedure for insemination consisted of the use of a glass or stainless steel inseminating tube which was inserted about an inch into the cervix before the semen sample was delivered. The amount of semen used for each insemination was one cc.

#### EXPERIMENTAL

Semen samples that were examined varied in the percentage of initial progressive motility from 10 to 100. More than 90 per cent of the samples ranged from 50 to 100 per cent in initial motility and only those were included since samples showing an initial progressive motility of less than 50 per cent may have been due to faulty technique in collecting the samples.

In table 1 the semen samples were divided into two groups, namely, those having an initial progressive motility of 50 to 70 per cent, and those having a motility ranging from 80 to 100 per cent. The pH values of the fresh samples and at various periods during storage, when stored at 35°, 40°, 50°,

TABLE 1

*Influence of storage temperature upon pH values of scmen samples grouped according to percentage of initial motility*

Storage period	Range in initial motility 50-70 per cent							
	Storage at							
	35° F.		40° F.		50° F.		70° F.	
	Samples	pH	Samples	pH	Samples	pH	Samples	pH
hrs.	no.	mean	no.	mean	no.	mean	no.	mean
Fresh	21	7.01	24	7.05	85	7.16	21	7.28
24- 28	2	6.78			22	6.54	13	6.31
48- 52	5	6.79	1	6.10	52	6.43	8	6.02
72- 76	7	6.77	5	6.70	19	6.22	1	6.39
96-100	10	6.90	10	6.90	28	6.25		
120-124	2	7.03	2	6.31	8	6.14		
144-148	5	6.66	7	6.59	9	6.21		
168-172			3	6.62	1	6.85		
192-196	8	6.85	7	6.67				
216-220	3	6.64	2	6.40	1	5.92		
240-244	1	6.97	6	6.54				
264-268								
288-292	9	6.85	5	6.34				
312-316			2	6.97				
336-340			1	6.18				
360-364	1	7.05						
384-388	9	6.70	4	6.97				
408-412	2	6.73	2	6.06				
432-436	1	7.14						
456-460								
480-484			1	6.69				
Range in initial motility 80-100 per cent								
Fresh	46	6.82	55	7.03	141	6.93	12	6.84
24- 28	6	6.53	1	6.64	52	6.38	7	5.96
48- 52	31	6.78	14	6.68	118	6.36	2	5.80
72- 76	6	6.59	6	7.05	37	6.11		
96-100	21	6.72	34	6.71	66	6.04		
120-124	9	6.62	4	6.72	29	6.03		
144-148	21	6.70	15	6.64	32	6.05		
168-172	2	7.32	3	6.86	9	6.44		
192-196	11	6.73	27	6.52	5	6.26		
216-220	5	6.64	4	6.39	2	6.21		
240-244	1	7.01	5	6.59	2	5.99		
264-268	2	6.78	1	6.88				
288-292	6	6.68	19	6.48				
312-316								
336-340	2	6.83	1	6.77				
360-364	4	6.57	1	6.24				
384-388			11	6.32				
408-412			1	7.11				
432-436	1	7.27						
456-460			3	6.11				
480-484			2	6.36				

and 70° F., are presented in the various columns of the table. All values reported represent means if more than one sample is represented. The

initial pH values for the group 50 to 70 per cent motility are 7.01, 7.05, 7.16, and 7.28, while the values for the group 80 to 100 per cent motility were

TABLE 2

*Influence of storage temperature upon motility of spermatozoa in semen samples grouped according to pH values*

Storage period	Range in initial pH—6.40–6.99							
	Storage at							
	35° F.		40° F.		50° F.		70° F.	
Samples	Motility	Samples	Motility	Samples	Motility	Samples	Motility	
hrs.	no.	mean	no.	mean	no.	mean	no.	mean
Fresh	47	81	44	76	125	80	17	75
24–28	12	60	2	60	62	61	10	35
48–52	33	53	15	44	107	48	7	13
72–76	10	47	9	39	37	38	1	10
96–100	25	39	24	37	67	28		
120–124	9	41	5	28	30	24		
144–148	20	30	16	29	35	20		
168–172	3	13	1	30	6	22		
192–196	13	26	19	29	5	20		
216–220	9	17	5	24	2	1		
240–244				17	2	5		
264–268	2	11						
288–292	1	19	13	18				
312–316								
336–340			2	6				
360–364	3	13	1	10	2	1		
384–388	2	10	8	7				
408–412	2	20	2	10				
432–436								
456–460								
480–484								

Range in initial pH—7.00–7.60

Fresh	20	75	37	78	117	67	16	67
24–28				30	21	50	10	28
48–52			3	35	69	39	3	13
72–76	7	44	6	35	14	33		
96–100	3	30	6	35				
120–124	9	29	20	24	26	30		
144–148	2	30	2	25	7	23		
168–172	8	30	9	27	8	23		
192–196			5	28	2	15		
216–220	8	21	15	17				
240–244	2	30	1	20	1	1		
264–268	1	20	4	28				
288–292			1	20				
312–316	8	15	10	12				
336–340			2	20				
360–364	2	10						
384–388	2	10						
408–412	7	10	6	14				
432–436	2	10						
456–460								
480–484			3	7				

respectively 6.82, 7.03, 6.93, and 6.84. It will be apparent from table 1 that storage at 70° F. causes a very rapid decrease in the pH value and that storage at 50° F. showed a rapid decline for the first 24 hours and a slower decline thereafter. At storage temperatures of 35° and 40° F. there was a very slow rate of decline in pH values for all periods of storage, the least shift occurring at 35° F. It is apparent that untreated semen tends to become more acid upon storage and that higher temperatures of storage tend to increase the rapidity of decline in pH. Semen stored at the higher temperatures reached lower pH values than that stored at 35° and 40° F.

Table 2 presents the data arranged according to the initial pH values of semen samples as measured in motility initially and after storage. In this table, the semen samples that ranged in initial pH values from 6.40 to 6.99 were grouped, and those that showed a range of 7.00 to 7.60 were grouped. In the first mentioned group, the initial mean percentages of progressive motility were 81, 76, 80, and 75 for the samples that later were stored respectively at 35°, 40°, 50°, and 70° F. The corresponding initial mean percentage of progressive motility for the semen samples showing a range of 7.00 to 7.60 pH value were 75, 78, 67, and 67. In every case there was a large drop in percentage of motility for samples stored 24 hours and a smaller relative decline during storage for longer periods. There was comparatively little difference between the storage temperatures 35° and 40° F. as to the keeping quality of the semen as measured by motility, although the lower temperature appeared to be slightly better. At 50° F. the rate of decline in motility was more rapid than at the lower storage temperatures and the survival was shorter.

Table 3 presents a study of semen samples during storage as measured by pH values. Initially the samples were divided into two groups, namely, those where the concentration per mm.<sup>3</sup> ranged from 1 to 999,000, actually from 8,000 to 999,000, and those which ranged higher, namely, from 1,000,000 to 1,999,000. It will be noted that the mean pH values for fresh samples of the lower concentration group showed 6.98, 7.15, 7.18, and 7.29 while for the higher concentration group, the respective values were 6.74, 6.76, 6.70, and 6.73. Storage at 70° F. always showed a marked drop in pH value during the first 24 hours and at slower decline thereafter. The 50° F. storage temperature showed the same general trend, but the drop in pH during the first 24 hours was not so great and the samples showed motility after longer storage periods. The samples stored at 35° and 40° F. showed the best keeping quality. Not only was the rate of decline slower, but the total decline in pH values was less than for the higher storage temperatures.

In table 4, the characteristics of fresh semen from the 11 fertile bulls are presented together with the fecundity of fresh and stored semen. The figures presented are mean values for volume, motility, pH value, and concentration for all samples of successive ejaculates obtained from the bulls

TABLE 3

*Influence of storage temperature upon the pH value of semen samples grouped according to concentration of spermatozoa per mm.<sup>3</sup>*

Storage period	Concentration of spermatozoa per mm. <sup>3</sup> 1-999,000							
	Storage at							
	35° F.		40° F.		50° F.		70° F.	
	Samples	pH	Samples	pH	Samples	pH	Samples	pH
hrs.	no.	mean	no.	mean	no.	mean	no.	mean
Fresh	40	6.98	55	7.15	178	7.18	23	7.29
24- 28	5	6.76			53	6.55	14	6.30
48- 52	21	6.96	13	6.91	121	6.57	9	5.99
72- 76	5	7.07	8	6.99	38	6.24	1	6.39
96-100	18	6.97	34	6.86	64	6.20		
120-124	5	6.76	3	7.11	20	6.09		
144-148	13	6.89	16	6.77	26	6.17		
168-172	2	7.62	4	6.89	6	6.51		
192-196	8	6.94	24	6.66	3	5.88		
216-220	5	7.13	3	6.59	2	6.02		
240-244	1	7.01	8	6.85	1	5.73		
264-268	1	6.75	1	6.88				
288-292	7	6.89	18	6.49				
312-316			2	6.96				
336-340	1	6.93	1	6.77				
360-364	2	6.93			1	5.92		
384-388	5	6.66	13	6.57				
408-412			2	6.04				
432-436	2	7.20						
456-460			2	6.12				
480-484			4	6.60				
Concentration of spermatozoa per mm. <sup>3</sup> 1,000,000-1,999,000								
Fresh	27	6.74	26	6.76	63	6.70	10	6.73
24- 28	3	6.31			20	6.07	6	5.91
48- 52	17	6.51	5	6.36	50	5.94	1	5.86
72- 76	8	6.45	4	6.68	17	5.93		
96-100	13	6.54	11	6.41	30	5.91		
120-124	6	6.63	4	6.28	18	6.08		
144-148	13	6.49	7	6.33	11	5.88		
168-172	1	7.04	1	6.51	4	6.51		
192-196	10	6.64	10	6.33	3	6.43		
216-220	5	6.45	3	6.20	1	6.30		
240-244			4	6.17	1	6.25		
264-268	1	6.81						
288-292	7	6.63	6	6.33				
312-316								
336-340	1	6.72	1	6.18				
360-364	3	6.48	1	6.24				
384-388	3	6.66	3	6.26				
408-412	2	6.73	1	6.14				
432-436								
456-460								
480-484								

during the study. The ranges for all samples were as follows: volume—0.5 to 12.0 cc.; motility—10 to 100 per cent; pH value—6.18 to 8.31; and concentration—8,000 to 1,997,000 mm.<sup>3</sup> (6). The semen used for insemina-

TABLE 4  
*Characteristics and fecundity of semen when fresh and after storage at different temperatures*

Name of bull	Breed	Average age years months	Volume	Fresh semen				Stored semen			
				Initial motility of spermatozoa	Initial pH value	Concentration of spermatozoa M/mm. <sup>3</sup>	In-semi-nations	Conceptions		Conceptions	
								mean	no.	no.	no.
Beta	*	3y 3m	c.c.	**	per cent	no.	no.	no.	no.	no.	no.
Bon Jamie	A	2y 0m	5.8	(43)	5.8 (24)	76 (24)	6.74 (24)	1008 (24)	5	5	5
Hankey	A	8y 0m	4.3	(79)	4.3 (43)	61 (40)	7.19 (43)	477 (43)	1	1	1
Benedict	G	3y 6m	4.3	(53)	4.3 (53)	79 (47)	6.78 (50)	1024 (50)	8	2	2
Marose	G	7y 1m	4.2	(82)	4.2 (44)	77 (40)	7.14 (43)	656 (43)	15	2	2
Caliph	H	2y 6m	3.1	(16)	3.1 (7)	70 (7)	6.79 (7)	700 (7)	2	1	1
Garion	H	1y 2m	4.6	(89)	4.6 (27)	84 (20)	7.06 (26)	692 (26)	29	1	1
Quanto	H	4y 7m	3.4	(38)	3.4 (17)	67 (12)	7.33 (17)	371 (17)	18	3	3
Quinter	H	3y 4m	4.8	(33)	4.8 (9)	73 (9)	6.70 (9)	967 (9)	9	1	1
Ton 86 C	J	2y 4m	5.8	(50)	5.8 (27)	77 (27)	6.88 (27)	515 (27)	14	2	2
Radiance	J	3y 3m	2.7	(30)	2.7 (14)	71 (9)	7.46 (14)	714 (14)	3	0	0
All ejaculates			4.2	(571)	2.4 (78)	65 (8)	6.70 (6)	833 (6)	21	1	1
					(273)	74 (237)	6.99 (266)	154 (266)	112	2	1
								154	734	11	1

\* A—Ayrshire.

G—Guernsey.

H—Holstein.

J—Jersey.

\*\* No. samples.

tion was taken from these samples. Thus the general character of the semen can be judged with reference to inseminations and conceptions. Samples were not stored successfully for insemination at 70° F. Mechanical difficulties in the storage chambers prevented the use for insemination of many samples stored at 35° F. Most samples of fresh semen were used for insemination within four hours from the time of collection. For this study, however, fresh semen was considered to be any sample that was stored less than 24 hours. While there were no inseminations reported in this study for the bull Bon Jamie, he was proved previously to be a fertile bull. Of the 154 inseminations of fresh semen, 112 resulted in conceptions, or a conception percentage of 72.7, that is, 1.375 inseminations per conception. These figures are more favorable than were obtained in a previous study with massage obtained semen (5) when 181 inseminations resulted in 107 conceptions. This was a percentage of 59.1 or 1.69 inseminations for each conception. The table shows the length of storage, storage temperature and inseminations and conception using stored semen. The numbers are small and are merely indications. Grouping the samples stored at 35° and 40° F. together, there were 13 inseminations which resulted in seven conceptions or 53.8 per cent. The 15 samples stored at 50° F. resulted in four conceptions, a percentage of 26.7. For all stored samples, 28 inseminations resulted in 11 conceptions or 39.3 per cent.

#### DISCUSSION

It has been shown previously in table 1 that variations in initial pH and in the percentage of progressive motility for the two groups of samples stored at various temperatures appeared to have little or no effect upon the pH value during storage. Since all samples upon which pH determinations were made showed motility of spermatozoa it may be concluded that initial variations in motility within the ranges studied did not have an appreciable effect upon the keeping quality of spermatozoa as measured. It appears also that slight initial variations in the pH values have no appreciable effect upon the pH values developed during storage. However, the rate of decline in pH values developed during storage apparently is proportional to the temperature of storage; namely, the lower the storage temperature the slower the decline in pH of the semen, with but little difference being exhibited between the samples stored at 35° and 40° F.

When the samples of semen were sorted according to pH values and tabulated in terms of percentage of progressive motility as in table 2, it was found that initially, the semen samples with higher pH values showed slightly lower mean percentages of motility, which is a confirmation of the data in table 1. When measured by motility, the initial variation of pH values appeared to have no significant effect upon the samples after storage, since there was an approximately equal decline in the percentage of motility for the groups stored at the same temperature.

A study of table 3 where the semen samples were grouped according to concentration of spermatozoa per mm.<sup>3</sup> indicates a definite relationship between high concentration and lower initial pH value. There was apparently no different effect after storage between the two general groups. Here again, the storage temperature seemed to be the critical factor as affecting the rate of decline in pH value with the lower temperatures of storage giving the best results.

From these studies it appears that fresh semen samples which possess a high degree of motility tend to have a high concentration of spermatozoa per mm.<sup>3</sup> and pH values slightly on the acid side. Based on a population study it is apparent that these factors are definitely associated with fecundity, since the values of the various criteria of the 182 semen samples used for insemination must have been close to the mean (table 4). While it is true that there are considerable variations in the characteristics of successive ejaculates of individual bulls (6, 7), the data in table 4 indicate that the mean values of the semen characteristics of the individual bulls varied little from the mean of the population. Since the volume and the motility of the fresh semen samples used for insemination were substantially the same (volume 1 cc., motility 70-90 per cent) it does not seem likely that any deleterious influence on the fecundity or fertilizing capacity of the semen could have been exerted by these factors in the cases discussed. Whether it is the pH value or the concentration of spermatozoa which influenced the fertilizing capacity of the fresh semen samples of the individual bulls is not apparent since there are no great differences in the percentage of conception between the individual bulls. Apparently, the mean concentration of spermatozoa from individual bulls may vary a maximum of 363,000 and the pH value 0.34 unit from the population mean, without having any appreciable effect on fecundity.

Obviously, the fecundity of stored semen was inferior to that of fresh semen. Since volume and concentration were usually the same, and motility was but slightly lower, the decrease in fecundity of the stored semen may therefore be sought in biological changes as manifested by a decline in the pH value. It has been shown in tables 1 and 3 that there is a rapid shift in the pH value of semen at various temperatures during storage and that the shift in pH was greater with the higher temperatures. The least change in pH took place during storage at 35° and 40° F. The fecundity of the stored semen corresponds to this finding. Combining the semen samples stored at 35° and 40° F., which were used for insemination, a conception percentage of 53.8 was obtained; whereas, those samples stored at 50° F. resulted in a conception per cent of only 26.7. It is reasonable to believe that it is not the pH *per se* which affects the fecundity, but that the shift in pH values of the semen is an expression of certain catabolic processes which take place during storage and which are detrimental to spermatozoa although the sperm cells are inactivated by lowering the temperature.

Decreased fecundity of stored semen has been observed by other workers.

Using motility as a criterion of survival, Hatziolos (9) found that the average length of life for bovine sperm was 27 hours when stored at 32°–37° F. and 22 hours when stored at 37°–43° F. The fecundity of semen stored at these temperatures was very low. Of 20 cows inseminated only two became pregnant, one conceived from a 24-hour-old sample and one from a sample 48 hours of age. Chabibullin (4) found great differences between the motility percentage and the conception percentage of stored sheep sperm. Thus the motility following 24 hours of storage was 67–70 per cent while the corresponding conception rate was 31–36 per cent. He believed that a storage temperature of 46°–50° F. was the important one for storing sheep sperm.

Although motility is necessary for fertilization and a lowering of motility is commonly accepted as being associated with reduced fertility, it would seem that motility should be used with caution as a single criterion of fertilizing capacity of stored semen. At best the microscopic determination of motility is only an estimate, and chemical changes detrimental to the fecundity of spermatozoa are likely to occur before any change in motility is manifested. In a study of the effect of storage upon semen, a determination of the motility of spermatozoa taken in connection with the decline in pH due to natural causes, may possibly offer a laboratory test that can be correlated with fecundity.

#### SUMMARY

Semen samples obtained by means of the artificial vagina from 11 fertile bulls were grouped according to motility, pH values, and concentration, and the effects of storage at 35°, 40°, 50°, and 70° F. were studied.

When the semen samples were grouped according to percentage of initial progressive motility, the higher group showed lower initial pH values but no apparent difference was noticeable between the groups during storage. The pH values declined during storage, the lower the temperature the slower the decline.

Grouping semen samples according to initial pH values indicated that the lower pH value was associated with higher progressive motility. During storage there was no apparent difference between the groups but there was a decline in percentage of progressive motility and the higher the temperature the faster the decline.

Semen samples, when grouped according to concentration of spermatozoa per mm.<sup>3</sup> showed that the higher concentration had the lower pH values. When stored at the various temperatures there was no difference in rate of decline in pH values between the groups.

Average semen characteristics for 11 fertile bulls, based upon several hundred samples, together with the number of inseminations and conceptions for both fresh and stored semen are presented. A total of 154 inseminations with fresh semen resulted in 112 conceptions, a conception percentage of 72.7, or 1.375 inseminations per conception. Grouping all stored

samples, 28 inseminations resulted in 11 conceptions, a conception percentage of 39.3, or 2.545 inseminations per conception. Fifteen inseminations from samples stored at 50° F. resulted in four conceptions or 26.7 per cent, while 13 inseminations from samples stored at 35° and 40° F. resulted in seven conceptions or 53.8 per cent.

A storage temperature of 35° F. was found to be the most advantageous for storing semen, based upon motility and pH values, since at that temperature the least change occurred. The fecundity of semen is best preserved when stored at the lower temperatures.

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## A COMPARISON OF THE RESULTS OBTAINED FROM INCUBATING BACTERIOLOGICAL PLATES AT 32° C. AND 37° C. ON THE BACTERIAL COUNTS OF MILK

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As a result of a ruling of the Boston Board of Health stipulating the use of 32° C. instead of 37° C. as the incubation temperature for making bacterial counts on milk, several series of parallel determinations were made using various grades of milk. Appropriate dilutions of the milk sample to be tested were made and two plates were inoculated from the same dilution blank. Tryptone-glucose-beef-extract milk agar (A.P.H.A. 7th edition) was poured into each plate and one of the plates incubated at 37° C., the other at 32° C., for forty-eight hours.

The data presented in tables 1 to 4 inclusive show scattered diagrams of the distribution of counts on the two media. Of particular interest are the results on grade A raw milk purchased for pasteurization and sale as grade A pasteurized milk. This milk is purchased on a basis of full premiums for milk with a bacterial count below 10,000 per ml. and a sliding scale of partial premiums for milk with bacterial counts between 10,000 and 25,000 per ml.

Obviously, if the incubation of plates at 32° C. materially increased the bacterial counts, a producer might receive partial premiums or perhaps no premium on milk which would have earned full premium if the plates had been incubated at 37° C. If the effect of such a change in the incubation temperature proved serious in this regard, it would leave but two alternatives; first, to change the premium bases in keeping with the increased counts, and second, to improve the quality of the milk (which is, of course, the objective of the Board of Health in changing the requirements). If, on the other hand, the increases in counts do not seriously affect the premium scale, or if the normal variations of the plating procedure overshadow the increases in counts induced by the lower incubation temperature, no change in the premium schedule need be made.

A study of the data in table 1 based on 253 samples of grade A raw milk as sampled at the receiving station leads to the general conclusion that the lower temperature of incubation has not seriously affected the premium schedule and that the effect is partly offset by the factor of normal variation of the plating procedure.

In table 2 are shown the results based on 777 samples of market milk which were pasteurized in the laboratory by holding for 33 minutes at 143.5° F. in a thermostatically controlled water bath. These counts are used by the field men as an important part of the quality control program. An arbitrary

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standard of 5,000 is used as a criterion of the need of immediate farm inspection. The extent to which these counts are increased by the lower incubation temperature has no effect on the price paid to the producer. An essential feature of any quality control program is the extent to which it enables the detection of the sources of poor quality milk. If the lower temperature of incubation more effectively throws a spotlight on the less desirable producer, the change in procedure would be beneficial to the central objective of the field man's efforts. It remains to be seen if the lower temperature of incubation will yield counts that check more closely with field inspection.

A study of the data in table 2 indicates that only a relatively small percentage of the counts were materially increased by the 32° C. incubation and that this effect was particularly noticeable in the samples in the higher brackets of counts. This, of course, suggests that the incubation of plates at 32° C. tends to accentuate the very conditions which a quality control program is designed to detect.

Tables 3 and 4 show the distribution of counts at 32° C. and 37° C. based upon samples taken from tank car shipments. In table 3 are presented the counts of the raw milk and in table 4 the "heat-resistant" counts on the same milk after pasteurization in the laboratory. From these data, as in previous tables, it seems logical to conclude that the lower temperature of incubation will not necessitate any material reorganization of the quality control program.

It should be emphasized, however, that all of these samples were "winter samples" collected between the months of January and March. Whether or not the bacterial flora of summer milk will be sufficiently different to alter the results cannot be determined from these data.

TABLE 1

*A comparison of the results of incubating 253 raw milk samples from grade A dairies at 37° C. and 32° C. Tabular values refer to the numbers of samples*

Ranges of counts at 37° C.	No. of samples	Ranges of counts at 32° C.						
		0 to 5,000	5,001 to 10,000	10,001 to 15,000	15,001 to 25,000	25,001 to 50,000	50,001 to 100,000	Over 100,000
0 to 5,000	129	93	26	6	1	3		
5,001 to 10,000	72	21	30	14	4	3		
10,001 to 15,000	26	2	5	10	7	1		
15,001 to 25,000	16	2	1		10	3		
25,001 to 50,000	9		1			5	1	1
50,001 to 100,000	1						1	
Total .....	253							

#### *Observations from table 1*

1. Of the 253 samples, 201 were in the full premium class (below 10,000) when the plates were incubated at 37° C. Of these 201 samples, 25 (12 per cent) were moved into the partial premium class (10,000 to 25,000) and 6 (3 per cent) were moved out of the premium class (above 25,000).
2. Of the 42 samples in the partial premium class (10,000 to 25,000) when the plates were incubated at 37° C., 27 (64 per cent) remained in the partial premium class,

- 10 (25 per cent) were moved into a full premium class, and 5 (12 per cent) were deprived of premium by incubating the plates at 32° C. In one of these the count at 32° C. was sufficiently increased to exceed the state legal standard of 100,000.
3. Due in part to normal variation in plate counting, one-third as many producers (12) were favored as were penalized (36) by the lower temperature incubation. Normal variation undoubtedly was responsible also for some of the penalties, that is, if the parallel plates had been incubated at the same temperature no doubt there would have been some "penalties."

TABLE 2

*A comparison of the results of incubating parallel plates from 777 laboratory pasteurized market milk samples at 37° C. and 32° C. Tabular values refer to the numbers of samples*

Ranges of counts at 37° C.	No. of samples	Ranges of counts at 32° C.				
		0 to 1,000	1,001 to 2,500	2,501 to 5,000	5,001 to 10,000	Over 10,000
0 to 1,000	618	519	68	21	6	4
1,001 to 2,500	64	24	22	11	2	5
2,501 to 5,000	33	9	5	10	6	6
5,001 to 10,000	22	5	1	4	5	7
Over 10,000	40	2			5	33
Total	777					

*Observations from table 2*

1. Of the 777 samples, 715 gave counts below 5,000 when incubated at 37° C. Of these 715 samples, only 29 (4 per cent) gave counts in excess of this arbitrary standard when incubated at 32° C.
2. A study of the data indicates that a greater percentage of those samples in the higher brackets (37° C.) were increased by 32° C. incubation than was observed among the samples in the lower brackets of counts (below 2,500).

TABLE 3

*A comparison of the results of incubating parallel plates at 37° C. and 32° C. from 140 samples of raw market milk from tank car shipments. Tabular values refer to the numbers of samples*

Ranges of counts at 37° C.	No. of samples	Ranges of counts at 32° C.			
		0 to 100,000	100,001 to 200,000	200,001 to 300,000	300,001 to 400,000
0 to 100,000	96	81	15		
100,001 to 200,000	33	6	24	3	
200,001 to 300,000	9		2	6	1
300,001 to 400,000	2	1			
Total	140				

*Observations from table 3*

1. Of the 140 samples, 129 conformed to the arbitrary standard of 200,000 or below when the plates were incubated at 37° C.
2. Of these 129 samples, only 3 (2 per cent) were caused to exceed the arbitrary standard by incubating the plates at 32° C.

#### CONCLUSIONS

1. Grade A milk (253 samples, raw milk counts): As a result of incubating plates at 32° C., 12 per cent of the samples were moved from full to

TABLE 4

*A comparison of the results of incubating parallel plates at 37° C. and 32° C. from 142 samples of market milk from tank car shipments pasteurized in the laboratory.*  
*Tabular values refer to the numbers of samples*

Ranges of counts at 37° C.	No. of samples	Ranges of counts at 32° C.				
		0 to 2,500	2,501 to 5,000	5,001 to 10,000	10,001 to 20,000	Over 20,000
0 to 2,500 .....	92	66	21	5		
2,501 to 5,000 .....	32	10	16	4	2	
5,001 to 10,000 .....	17		6	8	2	
10,001 to 20,000 .....	1			1		1
Total .....	142					

*Observations from table 4*

1. Of the 142 samples, 124 were below the standard of 5,000 when incubated at 37° C. Of these 124, there were 11 (9 per cent) which were caused to exceed the arbitrary standard when the plates were incubated at 32° C.
2. Of the 18 samples which were above the 5,000 standard when incubated at 37° C., 12 (66 per cent) were also above the standard when incubated at 32° C.

partial premium class, and 3 per cent were deprived of premiums. Of the samples in the partial premium class (at 37° C.), 12 per cent were deprived of premium. To offset this, however, 25 per cent were moved back into a full premium class. (table 1.)

2. Market milk (777 samples, heat-resistant counts) : On a basis of a 5,000 standard at 37° C., 4 per cent of otherwise acceptable counts were caused to exceed the standard when plates were incubated at 32° C. (table 2.)

3. Tank car samples (140 raw milk counts) : On a basis of 200,000 standard (37° C.), 2 per cent of otherwise satisfactory samples were caused to exceed the standard by incubating the plates at 32° C. (table 3.)

4. Tank car samples (142 heat-resistant counts) : On a basis of a 5,000 standard (37° C.), 9 per cent of otherwise satisfactory samples were caused to exceed the standard by incubating the plates at 32° C. To offset this, 33 per cent of the 18 samples which exceeded the 5,000 standard at 37° C. gave counts below that standard when incubated at 32° C. (table 4.)

5. The producers of grade A milk were not sufficiently penalized by the use of the lower temperature of incubation to warrant readjustment of the premium bases. It would seem more logical to correct this situation by intensified field work with the relatively few indicated producers, thereby benefiting by the change of methods, rather than loosening the requirements on all grade A producers.

6. Since the fundamental purpose of making most bacterial analyses on milk is to point the way toward improvement of milk supplies, it seems reasonable to conclude that the lower temperature of incubation facilitates the attainment of that objective.

## METHOD FOR DETERMINING LOSSES OF BUTTER FAT IN THE CREAMERY\*

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Creamery patrons and directors, and even some buttermakers, have only a vague understanding of what should be considered as a fair overrun to be obtained in the manufacture of butter. Some states, as Iowa, have laws specifying the maximum overrun that may be obtained. In such cases the creamery directors generally consider that a buttermaker who cannot obtain an overrun within a small fraction of the maximum legal overrun lacks in ability and they frequently look for another operator.

The current overrun trends have been studied at the Iowa Experiment Station for some time, with the cooperation of a group of carefully selected Iowa creamery operators. From the results thus obtained it has been possible to evolve a system by which it will be not merely possible to determine with a reasonable degree of accuracy the correct overrun, but also to locate various existing irregularities and losses of butter fat.

In this study each creamery cooperating with the Experiment Station submits samples from one churning monthly. These samples which are analyzed chemically, consist of a sample of the cream before pasteurization, another after pasteurization and one after the cream is in the churn. A sample is taken from the can rinsings, one from the buttermilk, one from the starter and three samples from the butter; the butter samples are taken from different locations in the churn. Forms have been prepared for recording results and a copy is mailed to each operator monthly giving results obtained. The items listed on this form with a sample set of determinations accompanies this report.

### *Calculation of loss and overrun data from chemical analyses and operator's reports.*

The entries to be made under "Analysis for Fat" and "Salt" need no explanation. The entries to be made under "Lbs. Cream in Vat When Sample is Taken" and "Lbs. Cream in Churn" require explanation.

If the can rinsings were not added to the experimental vat, the amount of cream weighed in, that is not placed in the vat, i.e., the rinsings, should be determined and subtracted from the pounds of cream received for the experimental churning.

Assume that 2,000 pounds of cream containing 40 per cent butter fat are received and that 100 pounds of can rinsings, testing 25 per cent butter fat,

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*Items listed on creamery record form, with a  
sample set of determinations*

Name of Creamery	Name of Operator	Date
<b>Analysis for fat</b>		
Butter	Butter	
Cream	Lbs. butter made	1064.62
vat	Lbs. butter sold	1058.62
churn	Lbs. fat in butter made	858.40
Can rinsings	Overrun	
Starter	From vat test	21.92
Buttermilk	From churn test	22.38
Lbs. can rinsings	Based on fat losses	21.92
Salt	Total per cent reduction, all losses	3.08
Analysis	Distribution of fat losses	
Lbs. in butter	Buttermilk	
Lbs. added	Lbs. buttermilk	1588.00
Per cent lost	Lbs. fat lost	8.92
Lbs. cream in vat when sample is taken	Lbs. butter lost	11.15
Lbs. cream received for vat	per cent total fat received	1.03
Less cream in can rinsings	per cent reduction in overrun	1.28
Plus lbs. neutralizer and rinsings added before pasteurization	Overweight	
Total lbs. cream in vat	lbs. fat lost	4.8
Lbs. cream in churn	lbs. butter lost	6.00
Plus lbs. can rinsings	per cent of fat received	0.55
Plus lbs. neutralizer and rinsings added after vat sample taken	per cent reduction in overrun	0.69
Plus lbs. starter	Composition	
Less lbs. evaporation during pasteurization	lbs. fat lost	6.71
Total lbs. cream in churn	lbs. butter lost	8.38
Lbs. fat	per cent of fat received	0.77
Can rinsings	per cent reduction in overrun	0.97
Starter	Miscellaneous	
Lbs. fat by vat test plus fat added	lbs. fat lost	0.99
Lbs. fat by churn test	lbs. butter lost	1.24
	per cent of fat received	0.12
	per cent reduction in overrun	0.14
<b>Remarks</b>		

are obtained. The amount of original cream in the can rinsings is then equal to  $100 \times 25/40 = 62.5$  pounds, which amount is subtracted from the 2,000 pounds of cream received.

If, on the other hand, the can rinsings are added to the cream received, and the sample of cream is taken before the rinsings and neutralizer are added, the pounds of water contained in the can rinsings must be included in the second column under "Lbs. Cream in Churn."

If the can rinsings are added to the cream received before the sample of cream is taken from the vat for analysis, the test obtained represents the

test of the original cream received + amount of water in the can rinsings. The amount of water in the can rinsings is determined by the following formula:

$$X = \frac{1}{2} [(R - C) + \sqrt{(C + R)^2 - 4CRt/T}]$$

This formula is derived from the following equations:

$$(C + X)T = CT_1 \\ Rt = (R - X)T_1$$

C represents lbs. cream received

R represents lbs. can rinsings

T represents test of cream + water in can rinsings

t represents test of can rinsings

X represents lbs. water in can rinsings

T<sub>1</sub> represents test of original cream

The following problem will serve as an illustration: "2000 pounds of cream have been received. One hundred pounds of rinsings testing 25 per cent butter fat are obtained. The rinsings are added to the cream before the sample is taken from the vat for testing and the cream plus the water in the rinsings contains 40 per cent butter fat. Determine the pounds of cream held in the vat at the time the sample is taken."

$$\text{Answer: } X = \frac{1}{2} \left[ (100 - 2000) + \sqrt{(2000 + 100)^2 - \frac{4 \times 2000 \times 100 \times 25}{40}} \right]$$

= 38.6 pounds of water.

This should be added to the amount of cream received. The vat, therefore, contains 2000 + 38.6 pounds of cream at the time when the cream sample was taken from the vat for testing.

Operators handling sour cream will frequently add the rinsings and then neutralize before the vat sample is taken for testing. In that case the vat will contain "Pounds cream received + Pounds water in rinsings + Pounds neutralizer solution," and the amount of water in the rinsings may be determined from the following equations:

$$(C + Y + N)T = CT_1 \\ Rt = (R - Y)T_1$$

$$\text{Then: } Y = \frac{1}{2} [C + N + R + \sqrt{(C + N + R)^2 - 4CRt/T}] - (C + N)$$

In this formula the symbols are the same as in the former formula except that Y stands for pounds of water in the can rinsings, N for pounds of neutralizer solution added and T for test of (cream + water in can rinsings + neutralizer solution).

When the cream is weighed in a weigh tank and the can steamings are run into the weigh can and are weighed with the cream, the total amount

of fat received is contained in the vat and the only water added is that which is required to rinse the spout and the amount in the neutralizer solution.

The pounds of cream in the churn are calculated by adding to the total amount of cream, as calculated for the vat, the amount of rinsings and starter added and subtracting the amount of water lost due to evaporation during the process of pasteurization.

The amount of water lost by pasteurization is determined by the following formula:

$$W = C - CT/T_2$$

W represents pounds of water lost

C represents total weight of cream in the vat when pasteurization is started

T represents fat test of cream before pasteurization

$T_2$  represents fat test of cream after pasteurization

*Illustration:* A vat of 2,400 pounds cream and 50 pounds of water tests 35 per cent fat. Determine the amount of water lost during pasteurization if the cream tested 35.3 per cent fat after pasteurization.

$$2,450 - \frac{2,450 \times 35}{35.3} = 20.8 \text{ pounds.}$$

The entries made under the heading "Lbs. Fat" require no explanation, except possibly the third column, "Lbs. fat by vat test plus fat added." By "fat added" is understood the fat in the starter or any other source of butter fat added after the vat sample has been taken.

The amount of buttermilk obtained is determined by the following formula by Mortensen<sup>1</sup>.

$$B = \frac{C(100 - 11 - T)}{100 - 11 - a}$$

B represents pounds of buttermilk obtained in free form

C represents pounds of cream churned

T represents test of the cream

a represents test of the buttermilk

The amount of butter taken from the churn less the amount allowed for overweight, is equal to amount of butter sold.

A fat content of butter above 80 per cent reduces the overrun from the theoretical 25 per cent.

The miscellaneous losses are determined from the following formula:

$$\text{Lbs. butter fat in vat} - (\text{Lbs. fat in butter} + \text{Lbs. fat lost in buttermilk}) = \text{Miscellaneous losses.}$$

The following problem will explain the method employed in determining the effect of each of the various fat losses on the final overrun.

<sup>1</sup> Mortensen, M. Management of Dairy Plants. The Macmillan Co., 1938.

"A vat of cream contains 2500 lbs. of 30 per cent cream from which are produced 915 lbs. of butter testing 80.6 per cent fat. The test of the buttermilk is 0.6 per cent and 4 lbs. of butter are allowed for overweight. Determine the per cent overrun."

The overrun determined by the regular method of figuring is equal to:

$$\frac{(915 - 4) - 750}{750} \times 100 = 21.46\%$$

That system of figuring is correct if the weights and tests are correct, but if errors have been made in weighing or testing the overrun should be determined from the losses occurring during the process of manufacturing. This is done in accordance with the method outlined in the following:

#### *Buttermilk Losses*

$$2500 \times \frac{100 - 11 - 30}{100 - 11 - 0.6} = 1669 \text{ lbs. buttermilk}$$

$$1669 \times .006 = 10 \text{ lbs. fat lost in the buttermilk}$$

$$10 \times 100/80 = 12.5 \text{ lbs. } 80\% \text{ butter lost in buttermilk}$$

$$2500 \times .30 = 750 \text{ lbs. fat in vat (no starter added).}$$

$$\frac{12.5}{750} \times 100 = \underline{\underline{1.67\%}} \text{ reduction in overrun.}$$

#### *Miscellaneous Losses*

$$750 - (915 \times 80.6 + 10) = 2.51 \text{ lbs. fat as Miscel. loss}$$

$$2.51 \times 100/80 = 3.137 \text{ lbs. butter}$$

$$\frac{3.137}{750} \times 100 = \underline{\underline{0.42\%}} \text{ reduction in overrun.}$$

#### *Composition Losses*

$$915 \times \frac{(80.6 - 80)}{100} = 5.49 \text{ lbs. fat}$$

$$5.49 \times 100/80 = 6.86 \text{ lbs. butter}$$

$$\frac{6.86}{750} \times 100 = \underline{\underline{0.91\%}} \text{ reduction in overrun.}$$

#### *Overweight*

$$4 \times \frac{80.6}{100} = 3.224 \text{ lbs. fat}$$

$$3.224 \times 100/80 = 4.03 \text{ lbs. } 80\% \text{ butter}$$

$$\frac{4.03}{750} \times 100 = \underline{\underline{0.54\%}} \text{ reduction in overrun.}$$

The final per cent of overrun

$$= 25 - (1.67 + 0.42 + 0.91 + 0.54) = 21.46\%.$$

Although the study to which this system has been applied is yet limited, the work so far seems to indicate that it has been of considerable value to some of the operators who have cooperated in the project.



# STUDIES ON THE SOURCE-ORIGIN OF ACTIVATED FLAVOR IN MILK

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## INTRODUCTION

Milk unduly exposed to radiation, either natural or artificial, will acquire a flavor defect called activated, sunshine, or burnt. This defect is to be differentiated from that referred to as oxidized flavor. Homogenized milk is known to acquire the flavor more readily than unhomogenized milk. Excessive or improper exposure of milk to artificial radiation is a frequent cause for occurrence of the flavor. The ultimate prevention of the development of the flavor, however, necessitates further information concerning its origin and method of development. In the study presented here, milk or milk products, and other substances were exposed for prolonged periods to radiation so as to accentuate the intensity of the flavor, and permit its ready identification and isolation. Reviews of the literature on the activated flavor of milk have been included in previous reports (4, 8). It was pointed out that the flavor originates with the protein fraction of milk, and it was indicated that the flavor which results from undue exposure to ultra-violet radiation is identical or very similar to the burnt or sunshine flavor caused by exposure of milk to sunlight. Radiation is known to cause destructive effects upon proteins in general and to produce disagreeable flavors and odors. Casein and lactalbumin have been shown to develop the typical activated flavor of milk. The present study is concerned with ascertaining the specific source of the flavor.

## EXPERIMENTAL PROCEDURE

Two general procedures were followed in determining the specific source of the activated flavor of milk. These were a study of the effect of radiation on the nitrogen distribution of milk, and an investigation of the effects of radiation on the flavor of milk protein hydrolysates, amino acids, and other selected materials.

The source of ultra-violet radiation was a Hanovia quartz mercury-vapor arc placed at a distance of 76.5 cm. above the various materials. The intensity of the radiation was approximately 700 microwatts per square centimeter per second, at the surface of the materials, and was maintained by means of a

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Hanovia-Englehard meter photocell circuit. The milk and other fluid products were irradiated after being placed in stainless steel pans 18 cm. square and 3 cm. deep. Two hundred ml. portions were usually used. Generally, the pans were placed on a platform which was given a steady rocking motion through eccentric drive, by means of an electric motor. The materials which were exposed in dry form were spread on parchment paper beneath the arc.

A recently recommended (5) semi-micro Kjeldahl method for the determination of the total-, casein-, albumin-, globulin-, proteose-peptone-, and non-protein nitrogen was used for a study of the effect of radiation upon the nitrogen distribution of milk. The milk was irradiated for 15 minutes so that it had a very strong activated flavor and odor.

Van Slyke amino nitrogen determinations also were made of excessively irradiated milk, skimmilk and cream, and compared with similar determinations of untreated samples. Since exposure to radiation was continued for four hours in these experiments, the materials were weighed before and after the exposure, and the moisture lost by evaporation replaced with distilled water. In some cases determinations also were made after heating the samples to 87.8° C. and cooling, a procedure which intensifies the activated flavor.

In order to determine whether riboflavin or other dialysable substances of milk have a role in production of the flavor, milk in collodion bags was dialysed with distilled water for 24 hours. Fresh milk in new bags was then placed in the water and the dialysing continued, this being repeated four times in order to increase the concentration of dialysable substances in the water. The riboflavin of milk is said to be about 90 per cent dialysable (2). The water had the yellow-green pigmentation characteristic of riboflavin. For further concentration this solution was condensed under reduced pressure to about 20 per cent its original volume. The temperature did not exceed 60° C. during the concentration. Samples of the original and of the concentrated solutions were exposed to the radiation and examined for flavor and odor. A sample of milk which had been dialysed for four days with daily changes of water was also exposed to the radiation and its flavor compared with that of an unexposed non-dialysed sample.

For preparation of the protein hydrolysates, casein was precipitated from skimmilk with acetic acid, washed with distilled water, suspended in 7 normal hydrochloric acid, and autoclaved for eight hours at 15 pounds steam pressure. After shaking the hydrolysate with charcoal and filtering, much of the hydrochloric acid was boiled off at reduced pressure. Water was added and the evacuation repeated several times. The acid hydrolysate and a neutralized portion of it were then exposed to the radiation. A pepsin hydrolysate of milk was prepared by acidifying 200 ml. milk to approximately pH 2.0 with hydrochloric acid, adding one gram of pepsin, and incubating for five days at 37° C. This hydrolysate in the acidic form and after being neutral-

ized was then irradiated. Bacteriological peptone and tryptone in dry form and dissolved in distilled water, and a commercial sample of soft curd milk prepared by enzymatic treatment (Enzylic Process) were subjected to action of radiation and compared with non-treated samples for presence of activated flavor and odor.

Amino acids, either chemically pure or of high quality,<sup>1</sup> in dry form, in distilled water, or in M/10 phosphate buffer at pH 7.0 were irradiated for one and one-half hours. Similarly, commercial granular gelatin in dry form and as a one per cent solution in distilled water was irradiated for two hours.

#### RESULTS OF EXPERIMENTS

##### *A. Effect of Radiation on the Nitrogen Distribution of Milk.*

Table 1 shows the nitrogen distribution in normal samples of milk. It also shows the nitrogen distribution in milk which had a strong activated flavor and odor caused by exposure to radiation for 15 minutes. Determinations made on three separate lots of milk failed to show any appreciable effect of radiation upon the total-, casein-, or albumin plus globulin-, nitrogen content of the milk. The proteose-peptone nitrogen was somewhat greater in the irradiated sample in two out of three examinations while the non-protein nitrogen was slightly less in all three cases. Although the exposure to radiation in these trials was prolonged enough to produce a very strong activated flavor and odor, the data fail to indicate any drastic effects upon the various nitrogenous fractions comparable with the observed effects on flavor. Previous reports have indicated no (1) significant influence of irradiation upon

TABLE 1  
*Effect of radiation upon the distribution of nitrogen of milk\**

Fraction	Nitrogen as per cent of the milk							
	Trial							
	I		II		III		Average	
	Unirradiated	Irradiated	Unirradiated	Irradiated	Unirradiated	Irradiated	Unirradiated	Irradiated
Total Nitrogen	0.5578	0.5488	0.5152	0.5118	0.5348	0.5404	0.5359	0.5337
Casein Nitrogen	0.4547	0.4457	0.4051	0.4004	0.4275	0.4345	0.4291	0.4269
Albumin + glo- bulin Nitro- gen .....	0.0611	0.0598	0.0644	0.0644	0.0639	0.0639	0.0631	0.0627
Proteose-pep- tone Nitro- gen .....	0.0140	0.0158	0.0178	0.0231	0.0154	0.0150	0.0157	0.0180
Non-protein Nitrogen .....	0.0280	0.0276	0.0284	0.0245	0.0280	0.0270	0.0281	0.0264

\* Milk exposed to radiation from quartz mercury-vapor arc for 15 minutes; intensity of radiation approximately 700 microwatts per square centimeter at surface of fluid.

<sup>1</sup> Obtained from Pfanzlischl Chemical Company.

the content of casein or albumin in milk, but has been found (7) to delay rennet coagulation of milk.

TABLE 2  
*Effect of radiation upon the Van Slyke amino nitrogen content of milk, cream, and skimmilk\**

Material	Milligrams amino nitrogen in 5 milliliters			
	Unheated		Heated	
	Unirradiated	Irradiated	Unirradiated	Irradiated
Milk**	1.01	0.96	1.01	1.04
	0.98	1.01	1.03	1.01
	1.02	0.96	1.02	1.02
Average	1.00	0.98	1.02	1.02
Cream**	1.31	1.25	1.35	1.29
	1.29	1.29	1.34	1.34
	1.26	1.21	1.35	1.32
Average	1.29	1.25	1.35	1.32
Skimmilk	0.89	0.87		
	0.89	0.88		
	0.88	0.88		
Average	0.89	0.88		

\* Products exposed to radiation from a quartz mercury vapor arc for 4 hours, intensity 700 microwatts per square centimeter at surface.

\*\* Determinations also made on these samples after heating to 87.8° C.

The results of Van Slyke determinations of amino nitrogen of excessively irradiated milk, cream, and skimmilk are shown in table 2. The data obtained from triplicate determinations indicate no appreciable difference in amino nitrogen content of exposed and non-treated samples. They show that even with the prolonged four-hour exposure to radiation there was no significant breakdown of the proteins that could be measured by the Van Slyke amino nitrogen method. This is especially significant, since during commercial irradiation milk is exposed for periods of only a few seconds at the most. Formol titrations made as preliminary work to the Van Slyke studies also failed to show any difference between samples of milk and cream exposed for long and short periods to radiation. These results indicate that the methods employed were not sufficiently sensitive to measure the protein breakdown believed responsible for the flavor production.

#### B. Effect of Radiation on the Riboflavin and other Dialysable Substances of Milk.

It has been indicated (3) that the burnt flavor resulting from the action of sunlight on milk has its origin in the casein-free albumin-free serum of the milk, and the coincidence between development of this flavor and the fading

in color of the serum pigment riboflavin was noted. Dilute and vacuum-concentrated solutions of riboflavin and other dialysable substances obtained from milk were exposed to the quartz mercury-arc radiation. The treatment resulted in a disagreeable flavor and odor and a bleaching of the riboflavin pigmentation. The flavor and odor definitely were not typical of the activated flavor and odor of excessively irradiated milk. Addition of this irradiated solution to normal milk resulted in an off-flavored product, but which was not similar to the activated flavor observed in milk unduly exposed to radiation.

Milk which had been continually dialysed for four days with daily changes of distilled water developed the typical activated flavor and odor observed in normal milk when similarly exposed to radiation. This shows that removal of a large proportion of the dialysable substances of milk does not alter its susceptibility to development of the flavor and odor.

#### *C. Production of Flavor and Odor in Protein Hydrolysates Exposed to Radiation.*

It seemed of interest to investigate whether the typical activated flavor and odor of milk could be produced by irradiation of partially hydrolysed protein, which would indicate whether unaltered protein is necessary for production of the flavor and odor. Irradiation of acid hydrolysed casein, either acidic or neutralized, caused a change in its flavor and odor. The flavor and odor were not typical of that of milk subjected to prolonged exposure to the radiation. Irradiation of the acidic or neutralized pepsin hydrolysate of milk caused a change in its flavor and odor. The flavor and odor were not typical of that of milk similarly treated. The appearance of the digest suggested extensive protein hydrolysis and was of such character that accurate flavor and odor comparisons could not be made.

Irradiation of bacteriological peptone in dry form for two hours resulted in an off-flavor that was not typical of that of irradiated casein or milk. The irradiation of bacteriological tryptone in dry form for two hours resulted in a burnt flavor which was slightly similar to the flavor observed when casein or skimmilk powder is irradiated under similar conditions. Irradiation of peptone or tryptone in distilled water for periods up to two hours caused an off-flavor and odor. The flavor and odor produced in the tryptone were slightly similar to but not really typical of that of milk exposed to radiation. Little or no similarity in flavor and odor of irradiated peptone and casein could be noted. No difference in flavor and odor could be observed between enzyme treated soft curd milk and non-enzyme treated milk when both were exposed to radiation. The slight protein hydrolysis of the soft curd milk was therefore without effect on the susceptibility of the milk to development of activated flavor and odor.

These studies show that irradiation of proteins which have undergone extensive hydrolysis causes changes in flavor and odor, although these

changes are not typical of those occurring in similarly irradiated casein or milk.

#### D. Development of Flavors in Amino Acids Exposed to Radiation.

Because the results obtained in the study of irradiated protein hydrolysates were inconclusive, attention was given to the effect of radiation on amino acids. The results obtained by irradiating the amino acids in dry form are given in table 3. The data show that cystine, cysteine, methionine,

TABLE 3  
*Effect of radiation from quartz mercury vapor arc on the flavor of amino acids in dry form\**

AMINO ACID	FLAVOR	
	BEFORE IRRADIATION	AFTER IRRADIATION
L-cystine, C.P.	No flavor	Burnt hair, strong sulfur
cysteine HCl, C.P.	Acid, smoky	Strong burnt, smoky
dl-methionine, C.P.	Sweet	Very disagreeable, similar to irradiated casein
dl-b-phenylalanine, C.P.	Sweet, medicinal	Sweet, medicinal
l-tryptophane, C.P.	Slightly bitter	Strong, similar to irradiated casein, sulfur-like
l-tyrosine, C.P.	No flavor	No flavor
l-histidine HCl, C.P.	Acid, salty	Strong, similar to irradiated casein, sulfur-like
glycine, C.P.	Sweet	Sweet
dl-alanine, C.P.	Sweet	Sweet
dl-serine, C.P.	No flavor	Slightly sweet
dl-threonine	No flavor	Slightly sweet
dl-valine, C.P.	Slightly sweet	Sweet
dl-norleucine	No flavor	Slightly sweet
dl-isoleucine, C.P.	No flavor	Nut-like
l-leucine, C.P.	No flavor	Burnt
l-aspartic acid, C.P.	Slightly acid	Slightly burnt
d-glutamic acid, C.P.	Acid	Slightly burnt, smoky
d-arginine	Bitter	Bitter
d-lysine 2HCl, C.P.	Acid	Bitter, nut-like
l-proline, C.P.	Sweet	Sweet
l-hydroxyproline, C.P.	Sweet	Sweet, bitter

\* Exposed to radiation 90 minutes, intensity approximately 700 microwatts per square centimeter at surface of material.

tryptophane, histidine, leucine, aspartic acid and glutamic acid developed at least a slight burnt flavor somewhat similar to that noted in irradiated dry casein or skimmilk powder. Irradiated cystine, methionine, tryptophane, and histidine possessed especially strong flavors which indicated that these amino acids may be important contributors to the flavor of casein or milk exposed to radiation. When a small amount of each of these four irradiated amino acids was mixed with unirradiated dry casein, the mixture was found to have practically the same burnt flavor as irradiated casein. The flavor of irradiated cysteine was also strong and suggestive of the flavor of casein and milk exposed to radiation.

Irradiation of the amino acids in distilled water and in phosphate buffer of pH 7.0 gave less conclusive results than were obtained by irradiation of the amino acids in dry form.

The development of peculiar odors and flavors is known to occur in various proteins exposed to radiation. Casein and albumin have been shown to possess the typical odor and flavor of milk similarly subjected to radiation. Because its different amino acid content made study of the source of activated flavor convenient, gelatin was included in these studies. Irradiation of dry granular gelatin for two hours failed to produce any appreciable amount of the typical burnt activated flavor observed in casein after similar treatment. Irradiation for two hours of a one per cent solution of gelatin in distilled water caused a slight change in flavor and odor, but the typical flavor and odor of irradiated casein and milk were not evident. Since these results are in contrast to previously reported findings (4), they were carefully repeated and verified.

Since gelatin either lacks or has a much lower content of tryptophane, methionine, and histidine (6) than does casein or lactalbumin it is tempting to hypothesize that this is the explanation for the absence of the typical activated flavor and odor when it is exposed to radiation. Both gelatin and casein have much lower contents of cystine than does lactalbumin.

#### DISCUSSION

The fact that ultraviolet radiation has destructive effects upon proteins and may produce unpleasant odors and flavors is in accord with the evidence that the activated flavor of milk originates with the proteins. However, the applied analytical procedures were not sufficiently sensitive to show that exposure of milk to radiation has a measurable destructive effect upon the proteins comparable with the effects on the flavor.

The failure of the irradiation of riboflavin and other dialysable substances of milk to produce any semblance of the typical activated flavor eliminates these substances as possible precursors of this specific flavor.

Irradiation of the various protein hydrolysates was carried out in order to discover whether the same flavor changes would result as are produced from the unaltered protein, which would indicate whether the flavor arises from certain molecular groupings. The inconclusive results obtained with the acid hydrolysate may have been due to removal of flavor precursors by the purification procedure necessary. The various hydrolysates have inherently such disagreeable odors and flavors that accurate flavor determination could not be made. Tryptone developed a flavor somewhat similar to the typical activated flavor, showing that this partially hydrolysed product of casein retains some of the flavor producing characteristics of casein.

The results of irradiation of the amino acids indicated that cystine, methionine, tryptophane, and histidine may be important contributors to

the activated flavor of milk. The importance of methionine, tryptophane, and histidine was also indicated by the lack of flavor production by irradiation of gelatin, in view of the low content of these amino acids in gelatin as compared to casein and lactalbumin.

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## ISOLATION OF SUBSTANCES RESPONSIBLE FOR THE ACTIVATED FLAVOR OF MILK

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### INTRODUCTION

The prevention of development of activated flavor in milk (7, 8) is at present limited to control of the conditions under which milk is exposed to artificial radiation, and avoidance of exposure of milk and milk products to solar radiation. The isolation and identification of the substance or substances responsible for activated flavor have yet to be completed in order that information on better control practices may be acquired. It is the object of this report to present methods for isolation and qualification of the substances responsible for activated flavor in milk.

The possible rôle of sulfur compounds as the cause of the activated flavor of milk has been suggested (2, 7). It was observed that exposure of a dilute sodium chloride elution product of casein to ultra-violet radiation resulted in an odor which resembled that of milk excessively irradiated and more particularly that which results from exposure of human skin to ultra-violet light. This was interpreted as suggesting the mobilization of SH bodies (2). Irradiation is known to affect the sulfur residues of proteins. For example, egg albumin irradiated by a mercury vapor lamp in an atmosphere of nitrogen gave a positive nitroprusside reaction indicative of the presence of sulphydryl groups (R-SH) (9). Irradiation of skin was found to result in formation of sulphydryl compounds (10).

Sulfur compounds are known to be responsible for various disagreeable flavors. The cooked flavor of milk heated to high temperatures has been associated with liberation of sulfides (5) and sulphydryls (6). It appeared significant that the temperature (about 76-78° C.) at which the cooked flavor of milk becomes evident and at which begins appreciable formation of sulfides and sulphydryls (5, 6) is near the temperature (82.2° C.) at which the activated flavor is definitely intensified when milk exposed to radiation is heated.

### EXPERIMENTAL PROCEDURE

The source of ultra-violet radiation was a Hanovia quartz mercury-vapor arc placed at a distance of 76.5 cm. above the milk or other selected materials. The intensity of the radiation was approximately 700 microwatts per square centimeter per second at the surface of the substances exposed,

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and was maintained by means of a Hanovia-Engelhard meter, photocell circuit. The milk or other fluids were exposed to the radiation after being placed in stainless steel pans attached to a platform given a steady rocking motion by an eccentric drive, attached to an electric motor. Materials which were exposed in dry form were spread on parchment paper beneath the arc.

The problem of isolating the compounds responsible for the activated flavor of milk was complicated by the fact that the measure of progress depended upon the senses of taste and smell. The methods employed in the work were necessarily chosen with this fact in mind.

*Isolation of Flavor Substance:* Various methods of isolating the flavor compounds were attempted. The method finally adopted utilized steam distillation, in the apparatus shown in figure 1. The apparatus consisted

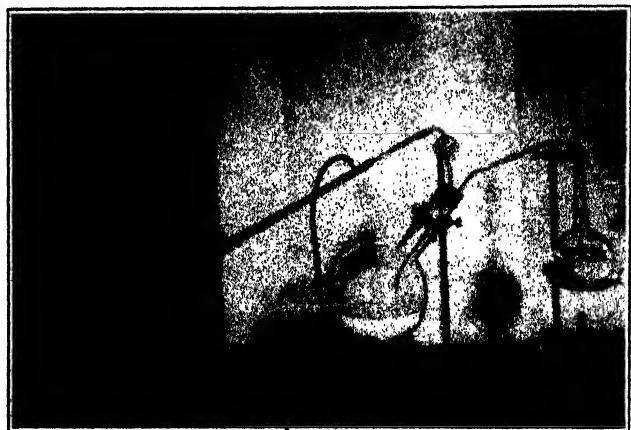


FIG. 1. Apparatus for separating from milk and casein suspensions by steam distillation, substances responsible for activated flavor.

of a pyrex flask, steam inlet and outlet tube, a Kjeldahl connecting bulb, condenser and adapter. Steam was prepared from distilled water. Fused glass and ground-glass joints were used where possible to minimize loss of volatile substances. While distillation of milk or cream was investigated, the use of an intensely irradiated (exposure, two hours) suspension of locust bean-gum-precipitated casein in distilled water (1) was found more satisfactory. The suspension could be distilled without difficulty of foaming, and the distillate possessed a strong activated odor. The casein assumes a colloidal suspension similar to that found in milk, when dispersed in water.

Approximately two liter samples of the casein suspension were distilled for a period of about 30 minutes. The flavor and odor materials recovered in the distillate were adsorbed with activated charcoal, followed by elution with ether. The distillate was collected beneath an aqueous suspension of

charcoal, which prevented loss of volatile substances. The charcoal and aqueous mixture were separated by suction filter. Prior to its use, the charcoal was washed with hot water, filtered, dried and extracted with ether in a Soxhlet apparatus. The charcoal was used for recovery of volatile material from several portions of various distillates of volatile substance. Practically all of the volatile odor material was taken up by the charcoal.

The ether used for elution was previously purified by shaking with concentrated sulfuric acid to remove alcohol, and with 10 per cent ferrous sulfate to remove peroxides, in order to prevent possible excessive oxidation of the volatile odor substances. The ether was then washed several times with water and distilled.

Elution of the flavor substances from the charcoal with the ether was conducted in a Soxhlet apparatus, using a ground glass joint between the ether flask and extraction tube. Only small volumes of ether were used.

The ether was removed from the elution product by distillation from a flask connected by a ground joint to a vertical Glinsky fractionating column. The ether escaping from the fractionating column was passed through a condenser into a receiver. It was impossible to remove the last traces of ether without excessive loss of the volatile odor material. The water which remained was frozen out and the ether solution decanted. The final ether solution having a volume of about 6-8 ml., obtained from around three kilograms of casein, had a very strong odor typical or identical to the characteristic odor of milk and milk proteins excessively irradiated. It was necessary to store the solution in a ground glass stoppered flask in a refrigerator to prevent loss of volatile odor material. When the flask was opened for a few seconds, the room was rapidly filled with the odor.

*Estimation of Sulphydryls in Milk; Steam Distillation:* A recently recommended procedure (3) was employed for the quantitative determination of the volatile sulphydryls of milk exposed to radiation. The method depends upon the formation of methylene blue from p-aminodimethylaniline by distillation of the sulphydryls into a hydrochloric acid solution of the reagent in the presence of added nitric acid solution of ferric chloride. The color formed is stable in light. In the reaction, each sulfur atom distilled as a sulphydryl enables the formation of one molecule of methylene blue. The details of the procedure as recommended (3) were followed except that steam and vacuum distillation were employed instead of flame heat distillation. Duplicate sets of distillation apparatus were used permitting simultaneous distillation of different samples of milk. In figure 2 is shown the equipment used for the steam distillation of the sulphydryls. The apparatus was made entirely of Pyrex tubing and flasks, the connections being either fused or of ground joints. The apparatus consisted of a distilling flask, a Kjeldahl connecting bulb, condenser and a vacuum connection receiving flask.

From fifty ml. of milk, forty-five ml. of distillate were collected in the

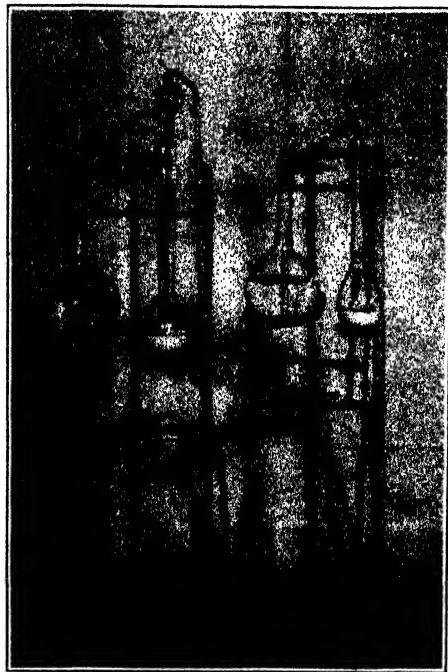


FIG. 2. Apparatus for separating from milk by steam distillation a distillate containing sulphydryls.

receiving flask beneath the surface of the reagents. Color comparisons of the distillate and standard methylene blue solutions by use of an Evelyn Photoelectric Colorimeter were promptly made.

The reagents were 0.5 per cent p-aminodimethylaniline in concentrated hydrochloric acid and a ferric chloride solution which consisted of 80 ml. of 10 per cent boiled-out nitric acid, 40 ml. normal ferric chloride and 40 ml. water. In all determinations, one ml. of the p-aminodimethylaniline solution, one ml. of a one to ten dilution of the ferric chloride solution, and three ml. water were placed in the receivers prior to distillation.

In order to limit the effect of heat in forming sulphydryls in the milk, the distillation was carried out as rapidly as possible without excessive foaming. The difficulty of foaming during distillation was largely prevented by addition of a few milligrams of lecithin to the milk. The time of active distillation was approximately 8 to 10 minutes.

As approximate color standards, suitable dilutions were prepared from methylene blue chloride (86 per cent dye content, National Aniline and Chemical Company). A concentration curve was established from readings promptly obtained using the photoelectric colorimeter. The concentration

of methylene blue formed in the distillates from the various samples of milk was determined from the colorimeter-concentration curve of the standard methylene blue solutions.

*Estimation of Sulphydryls in Milk; Vacuum Distillation:* Distillation under reduced pressure was also employed to minimize the effect of heat in forming sulphydryls from the milk and to determine the distillation temperature at which appreciable sulphydryl liberation would occur from milks given different degrees of exposure to ultra-violet radiation.

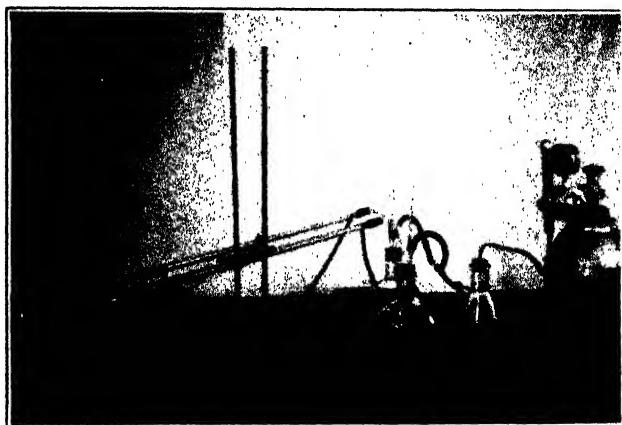


FIG. 3. Apparatus for separating from milk by vacuum distillation a distillate containing sulphydryls.

The equipment used for the vacuum distillations is shown in figure 3 and consisted of a source of nitrogen, alkaline pyrogallol wash bottle, distilling flask, condenser, receiver and vacuum flask.

Sufficient nitrogen was drawn through the milk to insure vigorous agitation and uniform heating. In the various determinations the total time of heating and distilling was limited to 35 minutes. Approximately 45 ml. of distillate from 350 ml. samples of milk were collected beneath the surface of the methylene blue reagents previously described, and the colorimeter determinations made with a Wilkens-Anderson KWSZ Photometer.

#### RESULTS OF EXPERIMENTS

##### A. Characteristics of the activated flavor concentrate.

The concentrate of material having an intense activated flavor prepared by ether elution of the adsorbate on charcoal from the distillate of casein appeared to be a homogeneous solution having a slight yellow color. Addition of a very small amount of this material imparted to milk a flavor and odor very similar to that of milk exposed to radiation. This indicated

the concentrate contained at least a large proportion of the compounds responsible for the activated flavor of milk.

When the material was held at -18° C. for 24 hours followed by several days' storage at about 4.5° C., a few small white crystals were formed. Analysis of the solution was not carried further than to show that it gave a strong nitroprusside test after treatment with potassium cyanide, and a strong sulfhydryl test with the methylene blue reagents after a few milligrams of zinc dust were added to the acid solution. Neither of these tests was positive without the reduction accomplished by addition of cyanide and zinc respectively, indicating the presence of disulfides.

*B. Liberation of sulfhydryls by steam distillation from milk exposed to radiation.*

Preliminary trials were made with skimmilk, milk and cream which had been exposed to the radiation produced by a quartz mercury-vapor arc for periods up to two hours. Rough color comparisons of the methylene blue formed in the distillates invariably indicated more sulfhydryl liberation from the milk exposed to radiation than from the milk not exposed to radiation.

The quantitative sulfhydryl-methylene blue procedure was applied to seven different lots of milk processed in a commercial, quartz mercury-vapor arc milk irradiator. The milk contained 400 U.S.P. units of vitamin D per quart. The raw milk was selected at random from mixed herd deliveries at the Department of Dairy Industry of the University. The samples were examined critically and found to possess only a slight degree of activated flavor.

The amount of sulfhydryls in the distillates of the various samples, as determined by methylene blue formation, is given in table 1. The values

TABLE 1

*Sulfhydryl content of distillates obtained by steam distillation from untreated milk and milk exposed to radiation from a quartz mercury-vapor arc lamp*

Lot No.	From milk exposed to radiation		From milk not exposed to radiation	
	Methylene blue chloride*	Sulfur	Methylene blue chloride*	Sulfur
	milligrams	milligrams	milligrams	milligrams
1 .....	0.116	0.0115	0.069	0.0068 *
2 .....	0.101	0.0100	0.081	0.0081
3 .....	0.093	0.0092	0.065	0.0064
4 .....	0.101	0.0100	0.030	0.0030
5 .....	0.103	0.0102	0.078	0.0077
6 .....	0.119	0.0118	0.087	0.0086
7 .....	0.114	0.0113	0.091	0.0090

\* Methylene blue chloride formed and its sulfur equivalent in 45 ml. distillate from 50 ml. of milk.

are expressed as methylene blue chloride and as sulfur. It may be observed that in every case appreciably more sulphydryl liberation was obtained from the milk exposed to radiation than from the milk not exposed to radiation. This indicates that even the limited exposure given milk in commercial units increases the lability of the sulfur of the milk and further that sulfur compounds may be responsible for activated flavor.

It was noted in a previous report (4) that gelatin failed to acquire significant amounts of the typical activated flavor when exposed to radiation. A two per cent solution of gelatin was exposed to mercury-vapor arc radiation for two hours. One hundred ml. of each of the untreated and exposed samples was steam distilled into the methylene blue reagents according to the procedure used for milk. No perceptible blue color could be noted after 45 ml. of either distillate was collected, thus giving a negative test for sulphydryl liberation.

Fresh egg white was diluted with distilled water and a portion exposed to the radiation for one hour. Steam distillation separately, of an untreated and an exposed sample into the methylene blue reagents resulted in greater blue color in the distillate from egg white which had been irradiated than in the distillate from untreated egg white.

In these experiments the gelatin developed practically none of the typical activated flavor or odor, whereas the egg white solution did develop the typical flavor and odor, providing further indication that sulfur may be involved in the development of activated flavor.

#### *C. Liberation of sulphydryls by vacuum distillation from milk exposed to ultra-violet radiation.*

Because of the small amount of sulphydryls liberated by the vacuum distillation procedure, and in order to accentuate the effect of the radiation, the milk used in these experiments was exposed to radiation for 90 minutes. The moisture which evaporated was replaced with distilled water. The milk possessed an intense activated flavor. The amount of sulphydryls obtained from the samples of milk by distilling at various temperatures is given in table 2, again expressed as methylene blue chloride and as sulfur. The results show that sulphydryl liberation occurred at appreciably lower temperatures from the milk exposed to the radiation, than from the milk not exposed to the radiation. At the distillation temperature ( $75^{\circ}$  C. and greater) which caused sulphydryl liberation from untreated normal milk, significantly more sulphydryl material was obtained from the milk exposed to the radiation. At the lowest distillation temperatures used ( $47$ – $58^{\circ}$  C.) no sulphydryl liberation from either samples of milk was detected by the test. These results are in agreement with those obtained using distillates derived from milk by steam distillation, and indicate that exposure to radiation increases the lability of sulfur of milk.

TABLE 2

*Sulfhydryl content of distillates obtained by vacuum distillation from untreated milk and milk exposed to quartz mercury-vapor arc radiation\**

Distillation temperature	Bath temp.	From milk exposed to radiation		From milk not exposed to radiation	
		Methylene** blue chloride	Sulfur	Methylene blue chloride	Sulfur
		<i>milligrams</i>	<i>milligrams</i>	<i>milligrams</i>	<i>milligrams</i>
77-79° C.	95° C.	0.132	0.0131	0.036	0.0036
74-75	90	0.071	0.0070	0.011	0.0011
68-70	85	0.037	0.0037	No blue color	
64-66	80	0.019	0.0019	No blue color	
58-60	75	questionable blue color		No blue color	
56-58	70	No blue color		No blue color	
47-48	60	No blue color		No blue color	

\* Irradiation for 90 minutes with quartz mercury vapor-arc.

\*\* Methylene blue chloride formed, and its sulfur equivalent in 45 ml. distillate from 350 ml. of milk.

#### DISCUSSION AND CONCLUSIONS

A method was developed for isolating and concentrating a highly volatile flavor and odor material believed responsible for the typical activated flavor produced in milk exposed to solar or artificial sources of radiation. Analysis of the material so isolated was not possible because of the small yield of product obtainable. Distillates from milk containing the flavor yielded a positive test for sulfhydryls, whereas the concentrate prepared according to the method described yielded only a strongly positive disulfide test. It is believed this is due to the oxidation of sulfhydryls during isolation of the flavor concentrate material. The chemical similarity of sulfhydryls and disulfides indicates the similarity of the flavor bearing products, and the rôle of sulfur bearing compounds in the source of the flavor.

The sulfur of milk exposed to ultra-violet radiation is more heat labile than that of milk not so exposed to radiation. The sulfur of egg white, which acquires a typical activated flavor when exposed to radiation is more labile than the relatively small sulfur content of gelatin which fails to acquire the flavor when exposed to radiation. This is further evidence of the rôle of sulfur compounds in the production of activated flavor.

Milk exposed to radiation may be differentiated from milk not exposed to radiation by use of a colorimetric test for sulfhydryls.

The increased lability of the sulfur of milk exposed to radiation also appears to be good evidence of the possible rôle of sulfur compounds in formation of the activated flavor. Although a limited heat treatment was necessary to remove sulfhydryls from milk exposed to radiation, the liberation occurred with less heating than was required for milk not so treated.

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## COMPOSITION OF GOAT MILK OF KNOWN PURITY

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There are more than three hundred registered goat breeders in Massachusetts. Information was received that the Goat Breeders' Association intended to introduce legislation pertaining to the sale of goat milk. It was deemed advisable to obtain samples of goat milk for analysis, the results of which would be of value to the legislative committee considering the proposed bill, which recommended standards for total solids and fat lower than those usually reported.

The reports of the Division of Livestock Disease Control of the Massachusetts Department of Agriculture made by the animal inspectors of the cities and towns in the state show the following number of goats in the respective years: 1934, 1,200; 1936, 1,598; 1937, 2,181; 1938, 2,527.

At this rate of increase there should have been 3,088 goats in Massachusetts at the close of 1939. It is probable that not all of the goats are necessarily reported by the local animal inspectors. For this reason Dr. Harric W. Peiree of the Division of Livestock Disease Control has estimated that at the close of 1939 there were nearly 4,000 goats in Massachusetts. Of this number only 246 have been "abortus tested," all of which were negative (1). Although this number of goats is altogether too small to produce even a minute portion of the two million quarts of fluid milk necessary for daily consumption as such, yet the number is sufficiently large to be of public health and legal significance.

A review of the literature of the past ten years shows considerable work pertaining to the composition of the fat of goat milk; but with one exception, nothing was found relating to variance in the solids, fat, proteins, etc., of goat milk.

König (2) quotes 111 analyses; Richmond (3) quotes König, Moser and Soxhlet, Fleishmann, Pizzi, Richmond, Piccardi, Steinegger, and Bosworth. Associates of Rogers (4) quote Frahm (5), giving the average of 326 samples by 18 investigators. These figures are shown in the table on p. 1098.

In March, 1939, after this study was started there appeared Technical Bulletin 671 of the United States Department of Agriculture on the subject of goats' milk by J. A. Gamble, N. R. Ellis, and A. K. Besley (6). That bulletin gives the results of a study extending over a period of three years. The goat milk used in that study was obtained from one herd of from twenty to thirty-five goats. The analyses reported are mostly of herd milk, but the data do not contain any figures relating to milk serum constants or to the phosphatase content.

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Source	Solids	Fat	Lactose	Proteins		Ash
				Casein	Albumin	
König	Highest	17.98	9.38	5.72	6.59	1.36
	Lowest	9.84	2.29	2.80	2.34	0.35
	Average	13.12	4.07	4.44	3.76	0.85
Richmond quotes						
König	14.29	4.78	4.46	3.20	1.09	0.76
Moser and Soxhlet	13.52	4.43	4.56	3.44	0.30	0.79
Fleishmann	14.50	4.80	4.00	3.80	1.20	0.70
Pizzi	13.25	5.35	3.60		3.64	0.67
Richmond	13.24	3.78	4.49		4.10	0.87
Piccardi	17.54	6.10	3.95	5.56		0.93
Steinegger	12.60*	3.25	4.80		3.92	0.63
Bosworth	.....	3.80	4.50		3.10	.....
Associates of Rogers quotes Frahm	12.86	4.09	4.20		3.71	0.78

\* This figure is quoted as 88.40 per cent moisture. It is obviously erroneous, since the sum of the fat, proteins, lactose, and ash is 12.60 and not 11.60.

In the absence of specific information relative to the source of the milk, the chemist making the analysis must assume that the sample may have been obtained from an individual animal and must base his conclusions as to whether the milk is normal or adulterated by comparison with analyses of milk obtained from individual animals rather than from herds. This is more important in dealing with goats' milk than with cows' milk because the goat herds are small, usually from three to five goats each, and if any abnormality exists in the milk of any one of these goats, it will not be effectively lost in the mixture as is usually the case with cows' milk where the herds are larger.

#### EXPERIMENTAL

The figures reported herein may be of interest since they represent milk obtained mostly from individual goats and include figures relating to the serum constants and also pertain to a study of the phosphatase content of goats' milk. Most of the goats were stall-fed with but little pasture. In a few instances alfalfa pastures were available. All but fifty of the samples used in this study were collected by Dr. G. L. Drury, a Veterinary Inspector with this Department. Each sample except one commercial sample was milked in the presence of an inspector of the Department, the inspectors personally delivering the samples to the chemists who made the analyses. The first samples were collected late in December, 1938, and it was then ascertained that there would be but little goat milk available until February. Samples, however, were obtained during January, February, March, May, June, July, August, and September.

The methods of analysis are as follows: Total solids were determined by drying a five-gram sample in a flat-bottomed platinum dish for two hours at the temperature of boiling water. The ash was determined by burning

with nitric acid the residue from the total solids. The fat was determined by the Babcock method. The total nitrogen, casein, lactose, freezing point, copper serum refraction, and acetic serum ash were determined by the official methods of the A. O. A. C. The phosphatase was determined by the Scharer method. (7) The calcium was determined upon the ash of the sour milk serum of the goat milk. This should give results as correct as if the determination were made upon the ash of the milk itself as was done on the samples of cow's milk. The method of analysis is as follows: After weighing the ash it was dissolved in dilute hydrochloric acid, the solution was nearly neutralized, sodium acetate and acetic acid were added, and the calcium was precipitated from the boiling solution by potassium oxalate. The precipitated calcium oxalate after filtering and washing was dissolved in hot dilute sulphuric acid and was titrated hot with permanganate.

Table 1 gives the summary of the analyses of these samples, the highest figure, the upper quartile, the median, the average, the lower quartile, and the lowest figure being given in each instance. Table 2 gives similar figures for samples of herd milk. The thirteen samples collected in the last week of December are included with the January samples, and May, June, and July samples are compiled together. No samples were collected during April. The samples collected in December and January were from seventy-seven individual goats and from one herd. The quantity given by each goat was small, and the average time since kidding was 6.24 months. Only a few goats had kidded recently, and many had been in lactation for more than a year. The milk collected during this period is characterized by high total solids. The goats supplying the samples collected in February showed an average of eight months since kidding, and the milk averaged in composition about the same as that of the samples collected in January. The lactation period for March averaged 2.1 months, yet the average composition of the milk varied only slightly from that produced in January or February. The samples collected during May, June, and July averaged much lower in total solids than the other samples. The average stage of lactation for these samples was 3.4 months, which was longer than that of the goats supplying the milk collected during March. Eighty-seven more samples were collected in August, the average period of lactation being nearly five months. This is considerably longer than the lactation period for the samples collected during May, June and July, and it averages nearly as long as that for those for January and February. Notwithstanding this advance in the lactation period, the solids content of the milk was on the average the lowest of any collected.

Table 3 gives the average composition reported by Gamble *et al.* (6, p. 14), together with the averages reported in table 1. Both figures show a high solids concentration in the milk produced in February and a low solids concentration in that produced in August.

TABLE I  
Summary of Analyses of Samples of Milk from Individual Goats

	Total Solids	Fat	Solids Not Fat	Lactose	Proteins	Casein	Ash	Freezing Point Depression	Copper Serum Refraction	Acetic Serum Ash
	%	%	%	%	%	%	%	%C.		
<i>Samples collected Dec. 21, 1938 to Jan. 26, 1939</i>										
Highest	21.06	9.80	11.90	5.50	6.08	.....	1.20	0.618	41.9	1.260
Upper quartile	15.50	5.75	9.80	5.05	4.39	.....	0.89	0.591	39.3	0.980
Median	14.72	5.00	9.53	4.80	3.88	.....	0.84	0.576	38.6	0.920
Average	14.50	5.08	9.42	4.78	3.99	.....	0.85	0.579	38.6	0.937
Lower quartile	13.12	4.25	8.89	4.45	3.38	.....	0.80	0.564	37.5	0.870
Lowest	11.70	2.95	8.07	3.80	3.15	.....	0.68	0.537	55.9	0.775
No. of samples	77	77	77	77	77	77	64	73	64	64
<i>Samples collected Feb. 1, to Feb. 28, 1939.</i>										
Highest	19.92	9.00	11.23	5.65	5.62	4.35	1.04	0.611	42.2	1.105
Upper quartile	16.04	6.05	9.94	5.20	4.13	3.18	0.90	0.586	39.7	0.985
Median	14.38	4.95	9.34	4.85	3.91	3.01	0.86	0.576	38.6	0.935
Average	14.56	5.13	9.43	4.87	3.97	2.98	0.85	0.577	38.8	0.943
Lower quartile	13.12	4.30	8.89	4.60	3.39	2.69	0.80	0.568	37.5	0.905
Lowest	10.72	2.40	8.82	4.20	3.03	1.98	0.70	0.550	36.3	0.815
No. of samples	62	62	62	62	62	54	62	62	62	62
<i>Samples collected Mar. 1, to Mar. 9, 1939.</i>										
Highest	16.50	6.45	10.45	5.50	4.61	3.68	0.92	0.587	41.6	1.080
Upper quartile	14.90	5.40	9.57	5.25	4.00	2.89	0.82	0.558	39.6	0.920
Median	14.24	5.00	9.14	5.10	3.62	2.65	0.78	0.573	38.8	0.880
Average	14.08	4.80	9.28	5.03	3.74	2.74	0.76	0.571	38.8	0.876
Lower quartile	13.06	4.20	8.75	4.75	2.55	0.70	0.563	38.1	0.830	
Lowest	11.44	2.45	8.37	4.45	3.09	2.28	0.60	0.547	36.2	0.780
No. of samples	35	35	35	35	35	34	35	35	35	35
<i>Samples collected May 23, to July 6, 1939.</i>										
Highest	16.62	6.65	9.97	5.10	4.67	.....	0.90	0.616	39.0	1.080
Upper quartile	13.06	4.05	8.92	5.00	3.78	.....	0.82	0.594	38.4	0.876
Median	11.74	3.55	8.24	4.60	3.42	.....	0.76	0.596	37.2	0.844
Average	12.24	3.79	8.45	4.66	3.34	.....	0.77	0.594	37.2	0.850
Lower quartile	11.38	3.20	8.02	4.45	2.76	.....	0.72	0.586	36.7	0.805
Lowest	10.30	2.70	7.60	4.15	2.44	.....	0.68	0.563	35.6	0.740
No. of samples	49	49	49	49	49	15	15	15	15	41

TABLE 1—(Continued)

	Total Solids	Fat	Solids Not Fat	Lactose	Proteins	Casein	Ash	Freezing Point Depression	Copper Serum Refraction	Acetic Serum Ash
	%	%	%	%	%	%	%	%C.		
Samples collected Aug. 1, to Aug. 31, 1939.										
Highest	15.80	7.00	9.46	5.20	4.20	..	0.88	0.646	38.4	0.960
Upper quartile	12.32	4.10	8.56	4.50	3.28	..	0.82	0.613	36.8	0.884
Median	11.04	3.15	7.99	4.30	2.91	..	0.80	0.588	36.3	0.840
Average	11.47	3.37	8.10	4.32	2.99	..	0.78	0.589	36.3	0.839
Lower quartile	10.44	2.50	7.68	4.15	2.67	..	0.76	0.588	35.8	0.790
Lowest	9.12	1.90	7.16	3.56	2.30	..	0.68	0.567	34.6	0.720
No. of samples	87	87	87	87	87	..	47	49	87	76
Samples collected Sept. 20, to Sept. 26, 1939.										
Highest	15.04	6.90	9.12	5.05	3.60	..	0.86	0.621	38.9	0.940
Upper quartile	12.92	4.50	8.72	4.80	3.43	..	0.82	0.598	35	0.900
Median	12.22	3.90	8.32	4.40	3.16	..	0.80	0.566	37.0	0.856
Average	12.29	3.98	8.31	4.49	3.16	..	0.79	0.589	37.0	0.857
Lower quartile	11.50	3.25	8.04	4.25	2.98	..	0.76	0.579	36.3	0.820
Lowest	10.94	2.60	7.68	4.05	2.66	..	0.72	0.568	35.5	0.768
No. of samples	25	25	25	25	25	..	25	25	25	25
Samples collected Feb. 7, 1940, to April 30, 1940.										
Highest	20.68	9.80	10.88	..	..	..	..	..	42.5	..
Upper quartile	15.62	6.40	9.34	..	..	..	..	..	39.6	..
Median	14.40	5.40	8.90	..	..	..	..	..	38.8	..
Average	14.54	5.55	8.99	..	..	..	..	..	38.8	..
Lower quartile	13.06	4.50	8.60	..	..	..	..	..	38.0	..
Lowest	10.72	2.90	7.38	..	..	..	..	..	36.2	..
No. of samples	104	104	104	..	..	..	..	..	104	..

TABLE 2  
*Analyses of Herd Samples of Goats' Milk*

Total solids	Fat	Solids not fat	Lactose	Proteins	Casein	Ash	Freezing point depression	Copper serum refraction	Acetic serum ash
%	%	%	%	%	%	%	°C.		
14.74	5.90	8.84	4.25	3.54	2.88	0.84	0.596	37.3	0.948
14.36	5.25	9.11	4.70	3.80	0.80	0.579	38.2	0.935	
13.58	5.00	8.58	4.55	3.46	0.80	0.591	37.1	0.900	
13.34	4.40	8.94	4.80	3.90	0.80	0.586	37.7	0.885	
12.92	4.35	8.67	4.50	3.05	0.82	0.579	36.7	0.904	
12.60	4.40	8.20	4.50	3.19	0.78	0.578	37.2	0.884	
12.54	4.00	8.54	4.70	3.39	0.78	0.610	37.7	0.850	
12.30	4.05	8.25	4.70	3.35	0.76	0.615	37.0	0.792	
12.30	3.90	8.40	4.35	3.18	0.76	0.585	37.2	0.840	
12.14	3.85	8.29	4.65	2.98	0.76	0.585	37.2	0.876	
12.08	4.10	7.98	4.30	3.15	0.78	0.583	36.4	0.840	
11.92	3.60	8.32	4.30	3.15	0.76	0.585	36.0	0.860	
11.80	4.05	7.75	4.25	2.83	0.76	0.590	36.0	0.876	
11.72	3.70	8.02	4.25	3.15	0.80	0.600	37.2	0.852	
11.58	3.30	8.28	4.50	3.09	0.76	0.600	37.2	0.820	
11.56	3.40	8.16	4.50	3.02	0.76	0.600	36.0	0.840	
11.52	3.50	8.02	4.25	3.00	0.78	0.586	36.4	0.840	
11.46	3.50	7.96	4.25	2.91	0.76	0.611	36.2	0.780	
11.40	3.50	7.90	4.35	2.71	0.74	0.611	35.9	0.780	
10.12	2.40	7.72	4.35	2.71	0.74	0.611	35.9	0.780	
9.94	2.50	7.44	4.25	2.71	0.74	0.611	35.9	0.780	
Samples collected February 7, 1940, to April 30, 1940, as follows:									
16.02	6.80	9.22						39.0	
15.36	5.80	9.56						38.9	
15.12	6.00	9.12						38.9	
14.92	6.00	8.92						39.3	
14.90	5.80	9.10						38.3	
14.56	5.60	8.96						39.6	
14.12	5.20	8.92						39.3	
14.10	5.20	8.90						39.0	
13.74	5.60	8.14						38.2	
13.60	4.60	9.00						38.6	
13.34	4.30	9.04						38.3	
13.30	4.50	8.80						39.0	
12.96	4.00	8.96						39.2	
12.56	4.40	8.16						37.7	
11.94	3.70	8.24						38.0	

TABLE 3

*Herd Milk Analyses of U. S. Department of Agriculture (6) and Average of Analyses Reported Here*

Month	Total solids	Fat	Solids not fat	Lactose	Pro- teins	Ash	Protein- fat ratio
	%	%	%	%	%	%	%
February	13.05	4.30	8.75	4.91	3.39	0.82	0.79
March	12.45	4.10	8.35	4.46	3.34	0.79	0.79
April	11.82	3.70	8.12	4.68	2.98	0.76	0.80
May	11.49	3.50	7.99	4.62	2.87	0.76	0.82
June	11.19	3.50	7.69	4.34	2.88	0.77	0.82
July	11.06	3.30	7.76	4.49	2.79	0.75	0.84
August	10.78	3.10	7.68	4.40	2.77	0.77	0.89
September	10.80	3.10	7.70	4.44	2.87	0.77	0.92
October	11.85	3.50	8.35	4.50	3.22	0.80	0.92
November	11.91	3.20	8.71	4.38	3.61	0.85	1.13
December	12.36	3.50	8.86	4.75	3.53	0.85	1.00
Average	11.71	3.50	8.21	4.55	3.10	0.79	0.89
<i>Average from Table 1, individual goats.</i>							
December and January	14.50	5.08	9.42	4.78	3.99	0.84	0.78
February	14.56	5.13	9.43	4.87	3.97	0.85	0.78
March	14.08	4.80	9.28	5.03	3.74	0.76	0.80
May, June and July	12.24	3.79	8.45	4.66	3.34	0.77	0.86
August	11.44	3.37	8.07	4.32	2.99	0.78	0.89
September	12.29	3.98	8.31	4.49	3.16	0.79	0.82

The analyses reported by Gamble show on the average a lower total solids and fat percentage than do those in table 1 and are less variable than those shown in table 2.

Most of the goats producing milk used in this study were of the Saanen and Toggenburg breeds. The analyses of the samples obtained from the Saanen and Toggenburg goats have been tabulated. During December, January, February, and March eighty samples were obtained from Toggenburg goats with an average solids of 14.18 per cent, fat of 4.97 per cent, and solids not fat of 9.21 per cent. During the same period samples were obtained from sixty Saanen goats, giving milk with an average solids of 14.24 per cent, fat of 4.95 per cent, and solids not fat of 9.29 per cent. During May, June, and July samples from thirteen Toggenburg goats were obtained with an average solids of 12.20 per cent, fat of 3.73 per cent, solids not fat of 8.47 per cent. During the same period samples with an average solids of 12.14 per cent, fat of 3.66 per cent, and solids not fat of 8.48 per cent, were obtained from twenty-seven Saanen goats. It appears from these figures that there is but little difference in the quality of the milk produced by these two breeds of goats. Several goat breeders stated that the Nubian goats gave richer milk than did the goats of the Saanen and Toggenburg breeds. Only eighteen Nubian goats were among those from which samples were obtained. The total solids of eight such samples collected during January, February and March varied from 15.32 per cent

to 18.40 per cent with an average of 17.13 per cent. Similar figures for the fat were 5.35 per cent, 9.00 per cent and 6.63 per cent respectively. The ten samples collected during June and August contained total solids from 11.38 per cent to 14.48 per cent with an average of 13.37 per cent and fat from 3.35 per cent to 5.50 per cent with an average of 4.45 per cent. These figures average higher than the results of the total samples collected, which for the 174 samples collected in January, February and March was 14.44 per cent total solids, 5.04 per cent fat and for the 136 samples collected in May, June, July and August was 11.74 per cent total solids, 3.52 per cent fat.

TABLE 4

*Comparison of Copper Serum Refraction and Serum Ash of Goats' and Cows' Milk*

Copper serum refraction	834 samples of cows' milk	174 samples of goats' milk collected Dec., Jan., Feb., Mar.	128 samples of goats' milk collected May, June, July, Aug., Sept.
<i>20° C.</i>	<i>Per cent of total samples</i>	<i>Per cent of total samples</i>	<i>Per cent of total samples</i>
34.0 to 34.9 .....	.....	..	3.1
35.0 to 35.9 .....	.....	1.1	20.3
36.0 to 36.9 .....	14.8	7.5	46.1
37.0 to 37.9 .....	35.8	24.2	20.3
38.0 to 38.9 .....	40.7	26.4	9.4
39.0 to 39.9 .....	8.1	22.5	0.8
40.0 to 40.9 .....	0.6	13.8	.
41.0 to 41.9 .....	.....	4.0	.
42.0 to 42.2 .....	.....	0.5	.....
<i>50% of samples between</i> .....	<i>37.4 and 38.4</i>	<i>37.6 and 39.5</i>	<i>35.9 and 37.1</i>
Serum ash	Sour serum ash, 371 samples of cows' milk	Acetic serum ash, 150 samples of goats' milk collected Dec. to Mar.	Acetic serum ash, 120 samples of goats' milk collected May to Sept.
	<i>Per cent of total samples</i>	<i>Per cent of total samples</i>	<i>Per cent of total samples</i>
0.72 .....	.....	0.7	.....
0.73 to 0.76 .....	31.4	1.3	7.5
0.77 to 0.80 .....	41.4	15.3	22.5
0.81 to 0.84 .....	17.7	17.3	21.7
0.85 to 0.88 .....	7.3	25.3	25.0
0.89 to 0.92 .....	1.4	18.0	15.0
0.93 to 0.96 .....	0.8	14.0	7.5
0.97 to 1.00 .....	.....	1.3	.....
1.01 to 1.04 .....	.....	2.7	.8
1.05 to 1.08 .....	.....	2.7	.....
1.09 to 1.12 .....	.....	0.7	.....
1.13 to 1.16 .....	.....	0.7	.....
1.17 to 1.20 .....	.....	0.7	.....
1.21 to 1.24 .....	.....	0.7	.....
1.25 to 1.26 .....	.....	0.7	.....
<i>50% of samples between</i> .....	<i>0.759 and 0.804</i>	<i>0.822 and 0.910</i>	<i>0.793 and 0.878</i>

As in the cows' milk the fat percentage is the most variable, the per cent of fat being higher during the winter months than it is during the summer. This seasonal variation is much greater in goats' milk than in cows' milk. The milk sugar does not differ greatly from that of cows' milk but averages somewhat lower. The proteins of goats' milk are, however, higher than those of cows' milk and are characterized by a lower casein content. The protein-fat ratio so useful in detecting skimming in cows' milk is of but little value when applied to goats' milk. The protein-fat ratio calculated from the figures reported by Gamble *et al.* (6) varies from 0.79 to 1.13. The figures reported here average from 0.76 to 0.89, with individual figures varying from 0.49 to 1.55.

Table 4 gives a comparison of the acetic serum ash of 270 samples of goat milk with the sour serum ash of 271 samples of cows' milk (8). In comparing these figures it should be understood that the acetic serum ash is 2 per cent lower than that of the sour serum. The same table also shows the comparison of the copper serum refraction of 302 samples of goat milk with that of 834 samples of cows' milk. Many years experience with these figures obtained from cows' milk shows but little seasonal variance. A recent compilation from records of this department shows an average copper serum refraction of 37.8 from 233 samples of known purity cows' milk collected in the winter, and of 37.6 from 385 samples of known purity cows' milk collected in the summer. The same figures from goats' milk show a marked seasonal variance and consequently in table 4 the results obtained from samples collected from December to March have been compiled separately from those collected from May to September. The serum ash of goats' milk varies more than that of cows' milk, and particularly in the winter is characterized by a higher figure. It is unusual to find serum ash figures above 0.88 in cows' milk but 40.1 per cent of the goat milk samples collected in the winter and 23.3 per cent collected in the summer gave sera with higher figures. It is unusual for the copper serum refraction of cows' milk to be above 40.0 or below 36.0, but 18.3 per cent of the goat milk collected in the winter gave sera above that figure as did 10.5 per cent of those collected in the summer. Few of the samples of goat milk gave copper sera with refraction below 36 in the winter, but 10.7 per cent of the samples gave sera of this character in the summer.

This is difficult to understand, unless it is due to a high albumin content in the winter and a low albumin content in the summer. Unfortunately, in this study the casein was determined only in the winter, and the same statement is also true to similar results reported by Gamble *et al.* (6). The average protein content of the 174 samples collected in the winter was 3.93 per cent and of the 127 samples collected in the summer was 3.07 per cent, representing a drop of 21.9 per cent which exceeds that of any other constituent except the fat. The non-casein proteins coagulable by heat have a greater influence on the concentration of goat milk serum than of cow

milk serum. Sixty-two samples were examined in February and March, 1940. The copper refraction plotted against the proteins of the serum shows a well-marked zone, the lower portion from 36.5 refraction 0.50 protein to 39.5 refraction 1.10 protein; the upper portion from 39.0 refraction 0.50 protein to 41.8 refraction 1.00 protein. The copper serum of each sample was heated for five minutes in a boiling water bath, filtered, and the refractive index again determined. The figures show a drop of from 0.8 to 3.8, average 2.08. Similar figures on known purity cows' milk show a drop of from 1.2 to 1.7 in copper serum refraction after heating. The freezing point depression in goats' milk is greater than in cows' milk. The amount of added water in cows' milk is computed from a freezing point depression of 0.55° C. The average of this figure for goats' milk varied from 0.57° C. to 0.59° C. The percentage of calcium in the ash is shown in table 5; it averaged 16.13. Four samples of herd milk from pure bred cows were obtained during July, and the calcium and ash were determined on each, using twenty-five-gram samples, each figure representing the average of three determinations. The percentage of calcium in the ash of the milk obtained from pure bred Jersey cows was 18.74; from pure bred Guernsey

TABLE 5

*Per cent of Calcium in the Acetic Serum Ash of Fifty Samples of Goats' Milk*

Lowest	13.11
Lower quartile	14.90
Median	16.13
Average	16.08
Upper quartile	17.02
Highest	19.95

cows, 18.49; from pure bred Ayrshire cows, 17.24, and from pure bred Holstein cows was 16.20.

Experiments were undertaken to ascertain if proper pasteurization of goats' milk would inactivate the phosphatase. Preliminary experiments indicated that if goats' milk were pasteurized at 142° F. for thirty minutes the phosphatase would be inactivated. In order to be sure that no idiosyncrasy of an individual goat could be responsible for opinions to the contrary, thirty-nine samples of milk obtained from individual goats were pasteurized in the laboratory. The Seharer modification of the phosphatase test was used, the reagents were prepared in the laboratory, and the incubation period was ten minutes. Of the thirty-nine raw samples collected one gave the deep blue color characteristic of the presence of phosphatase and similar in extent to the reaction given by raw cows' milk. Five samples gave a medium blue color, eleven gave a light blue color, ten a faint blue color, seven were indeterminate, and five gave negative reactions. These samples were pasteurized in the laboratory in test tubes at a temperature of 142° F. After five minutes at this temperature thirty-three gave negative phosphatase reactions, and in six samples the reaction was indeterminate.

After ten minutes of pasteurization thirty-six were negative, and three were indeterminate, but in fifteen minutes all were negative. Scharer (10) states regarding the modified method, "Chocolate drink, Vitamin D milk, condensed milk, goat milk and the like need no special treatment other than a control determination." He gives no figures relating to the thermal destruction of the phosphatase of goat milk.

In consideration of this phase of the subject the bacterial count may be of interest. During February, March and April, 1940 bacterial counts have been made upon goat milk. The goats were milked in the evening, the milk was refrigerated, and the plates were made the following day, corresponding to usual commercial conditions, using the new standard media of the American Public Health Association (11). Astonishingly low results were obtained. Of the 133 samples examined, one sample gave a count of less than 10, and the highest count was 85,000. The lower quartile was 131; the median was 575; the geometric mean was 586; the arithmetic mean was 1,130, and the upper quartile was 1,830. The geometric mean of the bacterial counts of 88 samples of raw certified milk examined during 1939 was 2,389.

Besley (12) has stated that the average bacterial count over a period of a year was 1,300 colonies per cubic centimeter. He collected samples aseptically from 23 goat udders and reports that 40 per cent of the milk did not show any bacteria, thus accounting for the low count of market goat milk.

#### SUMMARY AND CONCLUSIONS

Goat milk of known purity from 335 individual goats and 21 herds has been collected and analyzed. Determinations of solids, fat, total proteins, casein, lactose, ash, freezing point, and serum constants have been made. The per cent of calcium in the ash has been determined on fifty samples and has been compared with similar determinations on herd milk from pure bred cows. The rate of inactivation of the phosphatase of goat milk has been determined.

The watering and skimming of goat milk can be practiced to a greater extent with less chance of detection than is the case with cows' milk due to the greater variance in the serum constants of goat milk as well as of the freezing point.

The variation in the total solids and the fat is greater than is the case with cows' milk. In this work 85 per cent of the samples were obtained from Saanen and Toggenburg goats giving milk as nearly alike as could be obtained from Jersey and Guernsey cows, yet the individual variance is more marked than that found between Jersey and Holstein cows (9).

The goat milk produced in the summer months is inferior in solids content to the average market milk sold during the same period, and in order to obtain the same food value as that of cows' milk its consumption must be increased by 10 per cent.

Although the per cent of calcium in the ash of goat milk is substantially the same as in cows' milk, yet because of the higher ash content it is a valuable food when an excess of calcium is desired in the diet.

A study of the data indicates that the seasonal variation in composition is more marked than the variation with the lactation period.

The daily production per goat is small and in the 178 cases where a record was obtained it varied from 1 to 11 pounds with an average of 4.95 pounds per goat.

The phosphatase test is of little value in determining if goat milk is pasteurized as defined by law. If the phosphatase has not been inactivated the milk is raw milk, but it will be inactivated at pasteurization temperature considerably before the expiration of the legal holding time.

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# EFFECT OF THE PROPERTIES OF THE FAT AND OF THE FAT GLOBULE SURFACE ON LIPOLYTIC ACTIVITY IN MILK

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## INTRODUCTION

It may safely be assumed that all normal, raw, cow's milk contains lipase. It has been demonstrated that the velocity of lipase action on milk fat can be greatly accelerated by the so-called "activation" methods such as (1) homogenization (6, 13); (2) shaking (5, 10); and (3) cooling, warming and cooling (9). It is reasonable to assume that the quantitative effects obtained are dependent on (1) the amount of enzyme, (2) the properties of the fat, and (3) the properties of the interfacial layer.

This paper presents the results of some experiments which show that the properties of the milk fat as well as the conditions of the fat-plasma interface influence the lipolytic hydrolysis of milk fat.

## EXPERIMENTAL

### *Effect of fat content*

In order to determine the effect of concentration of substrate upon lipolysis, samples containing increasing amounts of fat were prepared by recombining the cream and skimmilk separated from fresh, raw milk. These samples were activated by cooling to 2° C., warming to 30° C. and recooling to 2° C. The increases in acidity of the plasma and of the fat were determined. The results presented are the differences in acidities obtained by subtracting the value of the pasteurized control after all samples had been held for 24 hours at 2° C. The acidity of the fat was determined by titrating 5 grams of fat with 0.05N alcoholic NaOH. The results are expressed in acid degrees, that is, cc. of 1N alkali per 100 grams of fat (7).

The data presented graphically in figure 1 show that the acidity of the total fat phase on the basis of the product increased with increasing fat content up to 35-45 per cent of fat, but that the acidity per unit of fat increased only up to about 8 per cent fat and from this point on the acidity per unit of fat slowly declined. The titratable acidity of the plasma phase, expressed as lactic acid, increased with increasing fat content up to about 6 per cent; however, at increasing levels of fat the titratable acidity of the plasma phase remained approximately constant. These results show the influence of the ratio of fat to plasma phase on the rate and character of milk fat lipolysis. At the higher levels of fat content the acid degree of the fat may be less, even though the total acids produced in the cream may be greater. At the fat percentage where the acid degree of the fat starts its decline, the acidity

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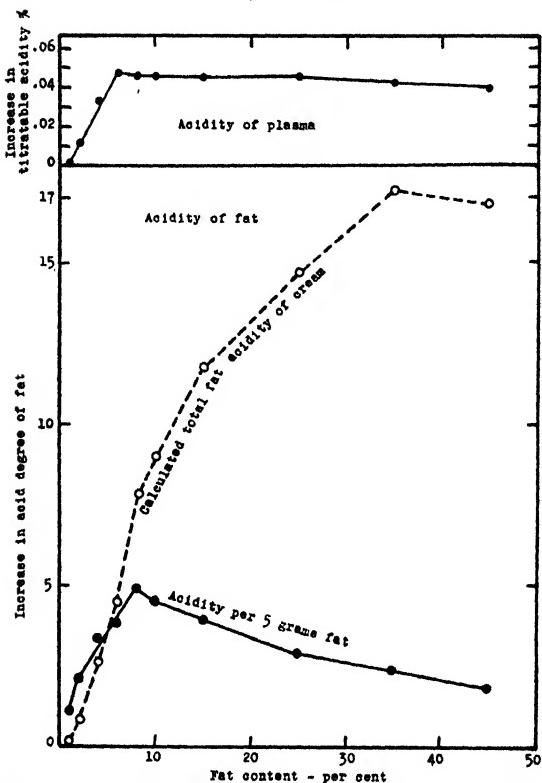


FIG. 1. Effect of increasing fat content on lipolysis after activation by cooling, warming and cooling. Values represent increase in acidity as a result of holding for 24 hours at 2° C.

of the plasma phase becomes constant. This interrelation indicates a depressing effect of the water-soluble fatty acids, produced by lipase activity, on the further activity of the lipase itself, and the establishment of a partition of the fatty acids between the plasma and the fat which reduces the hydrolysis of the glycerides which produce the water-soluble acids. The lipase continues to hydrolyze glycerides which do not produce water-soluble acids. It is intended to investigate this point further.

#### *Separation of milk fat fractions*

Chevreul (4) showed in 1823 that milk fat was a mixture of glycerides which he succeeded in separating into three fractions on the basis of their relative solubilities in alcohol. Much later Amberger (1, 2), using fractional crystallization from ether, alcohol and acetone, separated a number of fractions based on melting points, and claimed by this method to have

isolated small amounts of triolein and tristearin. Pizzi (11) separated milk fat into six fractions by removing the crops of crystals resulting from stepwise cooling. He found that the iodine number and Reichert-Meissl number of the fractions increased as the temperature at which the crystalline fractions were removed decreased.

The method of fractional crystallization was used to prepare a series of fractions of milk fat obtained from butter churned from fresh pasteurized cream. The water phase of the butter was separated from the fat by centrifuging in cups in a warmed centrifuge. The clear, supernatant oil was filtered through filter paper in a hot water funnel. The mixed glycerides were separated into a number of fractions by removing the crystals produced by stepwise cooling. The oil was first held for a week or two at a constant temperature of 30° C., the crystals formed were removed by filtration through a Büchner funnel, and the oil filtrate was then adjusted to a lower temperature for an additional period of holding, the second crop of crystals was removed, etc. A final oil was obtained in which no crystals were formed as a result of holding for several months at 4° C. Sharp separation of chemical entities is of course not produced by this single series of fractionations; however, the products thus obtained have sufficiently different properties to indicate trends. The yields of the various fractions are shown in figure 2.

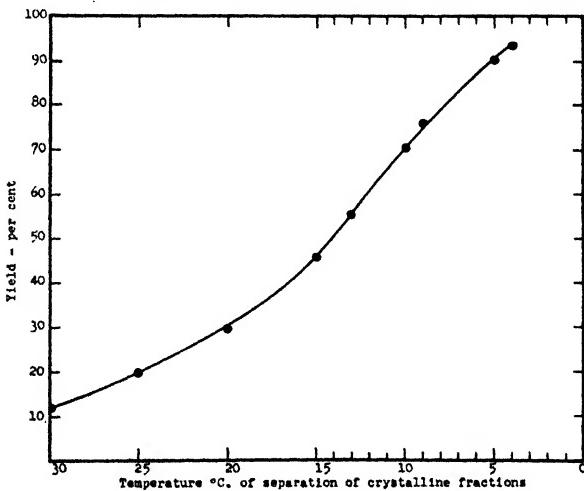


FIG. 2. Yield of crystalline milk fat resulting from stepwise cooling to a series of temperatures.

Attention should be called to the possibility that the presence or absence of at least certain portions of one fraction may affect the solubility of the other fractions in the residual mixture. Crystallization temperatures above 30° C., although not used in this instance, will produce small crops of crystals, as figure 2 indicates.

A number of the fat constants were determined on the various fractions using the A.O.A.C. (3) methods. The results are presented graphically in figure 3.

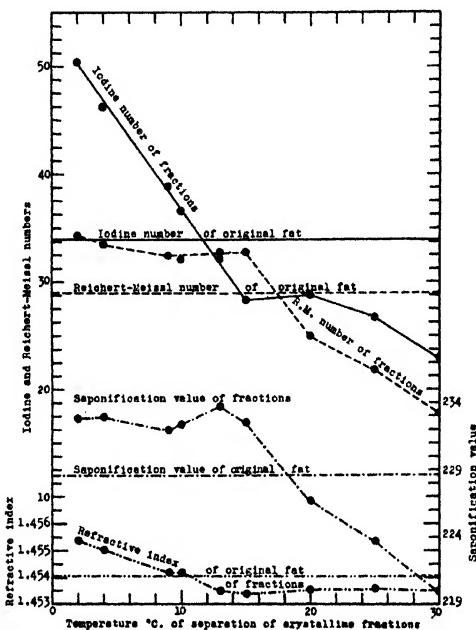


FIG. 3. Fat constants of various milk fat fractions separated by stepwise cooling.

The iodine numbers of the fractions obtained at temperatures lower than 15° C. show a marked increase, while the iodine numbers of the fractions separated above this temperature show a relatively slight decline. The data for refractive index show the same general trend. The curves for saponification value<sup>1</sup> and Reichert-Meissl number are similar in shape, the values tending to decrease in fractions above 15° C. and to remain constant in fractions obtained below this temperature. In general, figure 3 indicates that the fractions removed at the higher temperatures yield filtrates which are progressively richer in the shorter chain, low-molecular-weight acids and slightly richer in unsaturated acids down to 15° C. Below this temperature fractionation produced little change in molecular weight, but did markedly enrich the filtrate in its content of unsaturated fatty acids. The progressive crystallization of the various fat fractions is determined by their solubility in the uncrystallized glycerides remaining in solution at the various temperatures. It is apparent that the mutual solubility of the individual frac-

<sup>1</sup> The saponification values were kindly determined by Professor B. L. Herrington.

tions decreases with a lowering in temperature and the glycerides separate successively to the extent of their lowered solubility and the volume of solute available.

#### *Lipolysis of milk fat fractions*

Re-emulsified creams of 35 per cent fat content were prepared using pasteurized skimmilk and the following milk fats: unfractionated milk oil and fat fractions which were crystallized at 30, 25, 13, 10, and 4° C., and the oil remaining uncrystallized at 4° C. Re-emulsification was produced by homogenization. The attempt was made to produce fat emulsions in which the fat globules approximated in size those in normal milk. The adsorption layer on redispersed fat globules is of different composition and properties from the material present on the natural fat globules. We have used the term "resurfaced" to designate such globules. Each cream was diluted with portions from the same lot of raw skimmilk to produce a 3 per cent fat milk.

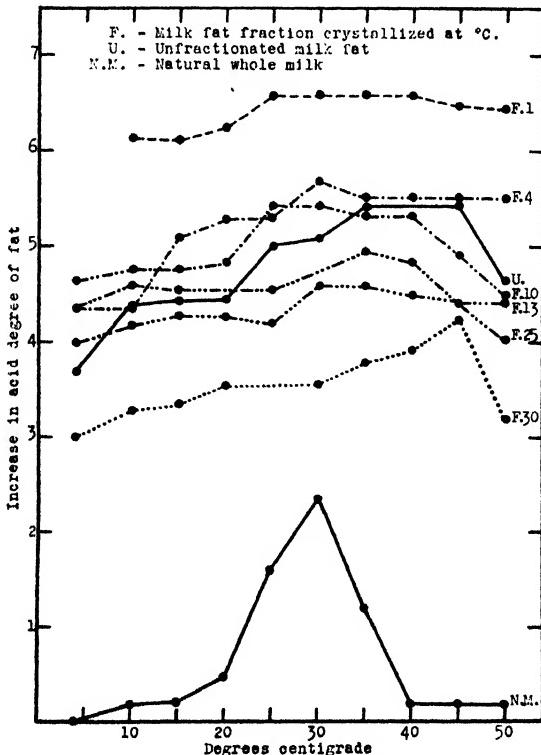


FIG. 4. Effect of preheating to various temperatures on the increase in acidity due to lipolysis resulting from 24 hours of holding at 20° C. Curves represent natural raw milk and reconstituted milk prepared from raw skimmilk and homogenized cream prepared from various fat fractions.

These milks, as well as a sample of the normal, whole, raw milk were cooled to below 5° C. and were then subjected to temperature activation. The milks were warmed gradually and aliquot samples were taken at 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50° C. and recooled. The acidities of the fats were determined after 24 hours of holding at 2° C. The increases in acidity as a result of holding are presented in figure 4. The data reveal that resurfacing of the fat as a result of emulsification with skimmilk renders the fat more susceptible to attack by lipase. Furthermore, since the lipase was already in the active state as a result of the resurfacing of the milk fat, it failed to respond in any appreciable degree to the activating influence of cooling, warming and cooling which exerts such a pronounced effect on normal, raw, whole milk with its naturally surfaced fat globules. The data in figure 4 show clearly that lipolysis was progressively greater with the fractions of progressively lower crystallization temperatures. About twice as much acid was produced during 24 hours holding at 2° C., in the fraction which did not crystallize at 4° C., as compared with the fraction which crystallized at 30° C. Attention should be called to the probability that the samples heated to 50° C. in figure 4 were partially inactivated by heat and oxygen, and this probably accounts for the slightly lower values obtained with some samples heated to that temperature.

*The conditions at the fat-plasma interface as influencing the temperature coefficient of lipase action in milk*

It was previously observed (13) but not reported at that time, in connection with cream separated at 85° F. from previously cooled milk, that the titratable acidity increased more rapidly the lower the temperature of holding. It was shown that the titratable acidity of homogenized milk increased with the temperature of holding. It was also shown (9) that the warming of cold, natural, raw milk to 30° C. greatly accelerates the lipolysis when the milk is recooled. However, the maximum effect does not appear unless the milk is cooled below 15° C. (59° F.). The above observations, and the data presented in figure 4, we interpreted as showing the marked contrast between natural and resurfaced fat globules.

A series of experiments was performed to show that the negative temperature coefficient for lipase activity was associated with the natural material on the surface of the fat globules, and that the expected normal positive coefficient was obtained when the fat was resurfaced. Reconstituted 35 per cent fat cream was prepared by homogenizing at 50–60° C. aliquots of the same pasteurized skimmilk with the following fat fractions: fraction crystallizing at 30° C., fraction not crystallizable at 4° C., pure triolein (m.p. –17° C.), pure trielaidin (m.p. 38° C.),<sup>2</sup> and unfractionated milk fat. These

<sup>2</sup> The sample of trielaidin was a preparation kindly furnished by Dr. Gordon Ellis of the Laboratory of Animal Nutrition of Cornell University.

creams, cooled to 37° C., were then diluted to 3 per cent fat with aliquots of the same raw skimmilk, also adjusted to 37° C. The five lots of milk described above, as well as one from the original whole milk taken at the time of milking, were placed in ice water. Samples were taken from each lot as the temperature fell to 25, 20, 15, 10 and 5° C. The samples removed were maintained at these same temperatures for 24 hours. In addition one lot of original whole milk was activated by cooling and warming, and aliquots were taken at these same temperatures during the second cooling.

Figure 5 shows the increase in the acidity of the fat as the result of 24 hours holding at the different temperatures. Lipolysis proceeded faster, the

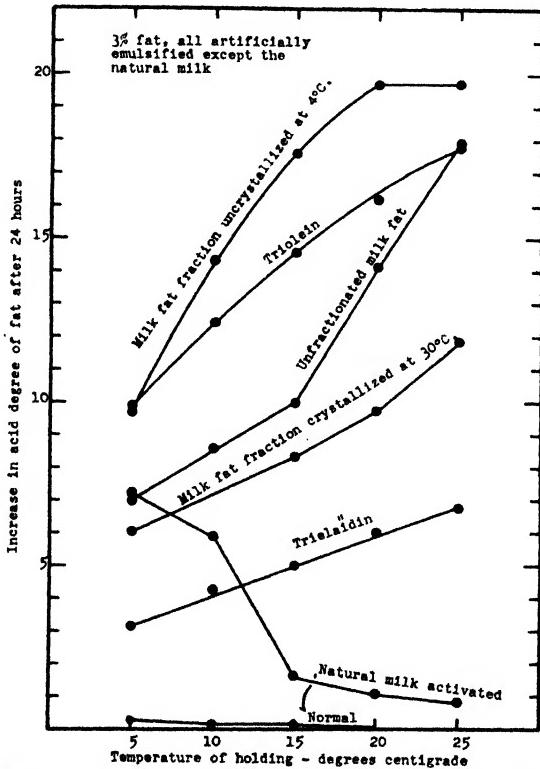


FIG. 5. Effect of temperature on lipolysis of normal surfaced and resurfaced milk fat and of fat fractions.

higher the temperature, in the case of all reemulsified fats, whereas lipolysis was faster the lower the temperature in the case of the fat globules with natural surfaces; this effect is shown most strikingly with the natural milk after activation. This experiment shows very clearly the difference in lipolytic susceptibility of fat globules with natural surfaces as contrasted with resurfaced fat globules.

As in figure 4, also in figure 5, lipolysis is greater the lower the melting point of the fat. A sharp break in the curve for the reemulsified, unfractionated fat occurs at 15° C. Near this temperature occurs a marked softening of the fat as shown by specific heat (12) determinations. Triolein and its isomer, trielaïdin, were used for comparative purposes because their melting points are more definite, and widely different, whereas the "melting" points of milk fat and to a lesser extent of milk fat fractions are more or less indefinite because they are mixtures. Lipolysis of the trielaïdin proceeded more slowly than of the triolein, as was expected from the difference in physical state. The curves for resurfaced fat globules indicate that the velocity of the lipolytic reaction and its final equilibrium are influenced by the physico-chemical properties of the fat.

*Effect of natural variations in fat, enzyme content, and the conditions at the fat-plasma interface on lipolytic activity*

The marked effect of the properties of the fat and the conditions at the fat-plasma interface raises the question as to the possibility of some of these variations occurring naturally. The following experiments were carried out to test this point. Milk from 9 individual Holstein and 8 individual Jersey cows was separated. The skimmilks were used to prepare standardized, 4 per cent fat milk, using in one series the same Holstein cream and in the other the same Jersey cream. In addition the lipolytic activity of the natural, unseparated milks was tested. The lipase in all milks was activated by cooling, warming and cooling, and the increase in acidity of the fat as a result of holding for 24 hours at 2° C. was determined. The results are expressed graphically in figure 6.

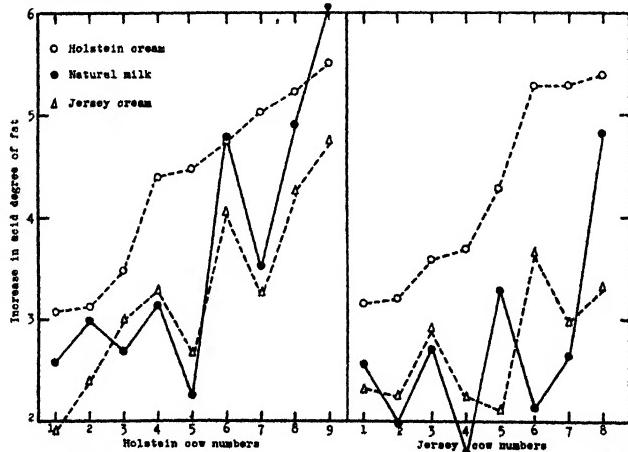


FIG. 6. Effect of raw skimmilk from various cows on the lipolysis of natural fat globules when natural whole and standardized milks were activated by cooling, warming and cooling. Increase in acidity as a result of holding for 24 hours at 2° C.

The samples are arranged in ascending order of the lipolysis of the milk standardized with Holstein cream. In general, the other two curves show a trend upward from left to right, indicating a variation in enzyme content in spite of the marked irregularities. Attention is called to the fact, however, that the lipolytic activities of the series of skimmilks standardized with Holstein and with Jersey cream do not exactly parallel each other, although all samples contain the same amount of fat, the samples in each series containing the same kind of fat and fat globules, and the pairs made from the same skimmilk contain the same amount of lipase. The chance differences between the two kinds of fat or cream probably explains why more lipolysis occurred in the Holstein fat. We are inclined to explain the irregular variations between the milks standardized with Holstein and Jersey creams as being due to differences in the interrelations of the various factors at the fat surface. The lipolytic activity of the natural milk when activated shows additional dissimilarities from the curves for the standardized milks. This result should be expected because of the additional influence of variations in fat content (substrate concentration) as well as the effect of the variability in properties of the individual fats and their surfaces.

#### CONCLUSIONS

The rate of lipolysis of milk fat is influenced by the activation procedure, the amount of enzyme present, the fat (substrate) concentration, the fat-plasma ratio, the physico-chemical properties of the fat, and the conditions of the fat-plasma interface.

Cooling, warming and cooling is effective in increasing lipolytic activity in raw cream as well as in raw milk.

Total lipolytic action increases with fat content up to 35-45 per cent, but acidity per unit of fat and the acidity per unit of plasma increases with fat content up to 8-10 per cent of fat and then remains constant or decreases.

Crystalline fractions separated from milk fat by stepwise cooling show a broken trend toward higher iodine, Reichert-Meissl and saponification numbers.

The lower the temperature required for crystallization of the fat fractions, the greater the increase in acidity when used as a substrate for milk lipase, which indicates that the rate of lipolysis is dependent upon the melting point of fat or upon the degree of solidification of fat at a given temperature. The degree of solidification is determined by the mutual solubility properties of individual glycerides of which milk fat is composed.

Lipolysis of milk fat is accelerated by resurfacing the fat globules.

Resurfaced fat globules show no further increase in lipolysis due to cooling, warming and cooling.

The rate of lipolysis of resurfaced fat globules increases with increasing temperature (showing a normal temperature coefficient), whereas the rate

of lipolysis of fat globules with the original normal surface increases as the temperature is lowered (showing a negative temperature coefficient).

The experiments demonstrate the influence of the conditions at the fat-plasma interface on lipolytic activity in milk and cream.

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# INACTIVATION OF MILK LIPASE BY DISSOLVED OXYGEN

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## INTRODUCTION

It has long been known that certain heavy metal salts inactivate or "poison" many enzymes. Davies (1) found that dissolved copper, in concentrations of 2 and 10 parts per million, as well as some other metal salts, retarded the action of milk lipase. He interpreted his results as indicating the susceptibility of the lipase to break down under conditions of low active oxygen concentration in the presence of an activating catalyst such as heavy metal salts. He correlated the depressing effect of metals on milk lipase with their catalytic power in inducing oxidation of milk fat, and suggested that destruction of lipase was catalyzed by heavy metal salts according to their varying powers of activating oxygen. Herrington and Krukovsky (4) reported that 0.2 and 0.4 parts per million of copper reduced lipase activity by about 20 per cent and that smaller amounts had less effect. Hellerman, Perkins and Clark (3) found that urease was inactivated by oxygen in the presence of dissolved copper.

We performed experiments to test Davies' explanation of the destructive action of copper on lipase and to determine whether dissolved oxygen must be present for this destructive action to occur in normal milk. The hypothesis that lipase is inactivated by dissolved oxygen as well as by heat has a bearing on the fact that most of the lipolytic activity in milk is inhibited by heating to relatively low temperatures. To test this idea, a comparison was made of the heat stability of lipase in normal and in deaerated milk, with and without added copper.

## EXPERIMENTAL

The importance of dissolved oxygen as one of the primary reactants in the inactivation of milk lipase in the presence of dissolved copper was demonstrated. Whole raw milk was divided into two parts and one part was deaerated. The oxygen-free milk was produced by subjecting milk previously heated to 46° C. to low pressure so that the temperature was caused to fall about 20° C. by evaporation of water (2, 7). A series of aliquots containing increasing amounts of dissolved copper (added as copper sulfate) was prepared using each lot of milk. After preparation the lipase in the aliquots was activated by the cooling, warming and cooling method described by Krukovsky and Herrington (5). The milk was held for various periods of time at 2° C. and the increases in acidity of the fat were determined by titration. The results presented are the differences in acidities

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obtained by subtracting the value of the pasteurized control after all samples had been held for various times at 2° C. The acidity of the fat was determined by titrating 5 grams of fat with 0.05N alcoholic NaOH. The results are expressed in acid degrees, that is, cc. of 1N alkali per 100 grams of fat (4).

The results of a typical experiment are presented in figure 1. The destruction of lipase in the presence of dissolved copper was almost entirely

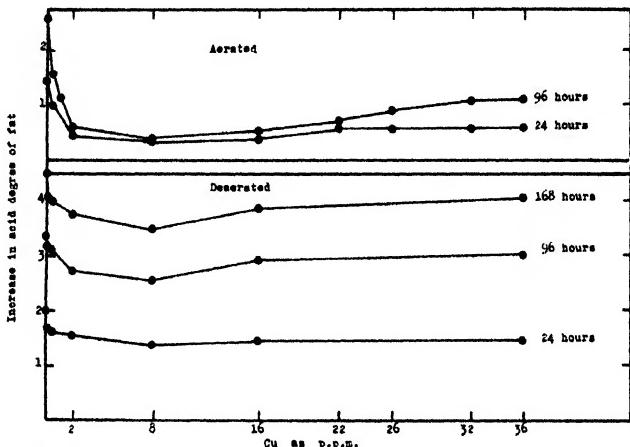


FIG. 1. Effect of increasing amounts of copper on the lipolysis of normal (aerated) and deaerated whole raw milk held at 2° C. for various periods of time.

prevented by the removal of the dissolved oxygen, whereas in the presence of dissolved oxygen the destruction increased with increased copper content up to 2 to 8 mg. per liter. This experiment confirms Davies' general hypothesis and shows that dissolved oxygen is necessary for the destructive action. There is some indication that with the larger amounts of copper a second factor enters which produces a slightly greater acidity of the fat with time. The data also indicate that in the absence of added copper more lipase activity was found in the deaerated than in the normal milk. This can be interpreted as an indication of the presence of a small amount of catalytically active copper or other catalyst in normal milk, or merely that the destructive effect of dissolved oxygen is proceeding normally during the measurement of lipase action.

The slight decrease in development of acid in the deaerated samples upon the addition of the smallest amounts of copper indicates that a trace of oxygen still remained in the milk. With this exception, figure 1 shows that in the absence of dissolved oxygen, dissolved copper exerts no destructive action on lipase even when present in concentrations up to 36 parts per million.

The effect of the removal of oxygen in lessening the destruction of lipase by heat was demonstrated by suitable experiments. The results of one of these are presented graphically in figure 2. Aliquots of raw whole milk,

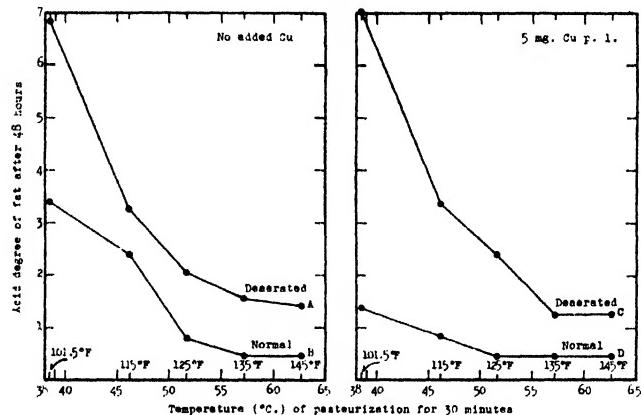


FIG. 2. Effect of temperature on the inactivation of lipase in normal and deaerated milk, with and without added copper. Lipase activated by cooling-warming-cooling, and activity measured by the increase in acidity of the fat as a result of holding for 48 hours at 2° C.

deaerated and normal, and with and without added copper, were heated for 30 minutes at various temperatures. Markedly less destruction of the lipase took place when the oxygen-free samples were heated, as contrasted with the normal milk which had not been deaerated; compare curves A and B. The difference is even more marked when the experiment was performed with added copper; compare curves C and D. Curves A and C are almost identical and confirm our other results by indicating that dissolved copper exerts no destructive action on milk lipase in the absence of dissolved oxygen. A comparison of curves B and D shows that in the presence of dissolved oxygen, copper exerts an accelerating effect on the destruction of lipase.

#### DISCUSSION

Following the lead given by Davies, it was shown that the lipase in normal milk may be inactivated by a reaction with dissolved oxygen in which dissolved copper acts as a catalyst. The results obtained indicate that normal milk contains enough dissolved copper or other catalyst to cause the inactivation of lipase by dissolved oxygen at relatively low temperatures of heating. The relatively low temperature of destruction of lipase in normal milk is the resultant of two processes: (1) the effect of the increased temperature in accelerating the inactivation of lipase by dissolved oxygen, and (2) the general destructive effect of heat, which is typical of most true enzymes.

Variations in the amount of dissolved oxygen and in the copper content of the milk, as a result of contact with equipment containing copper, may account for some of the variation in results obtained on the inactivation of lipase when milk is subjected to intermediate temperatures (100 to 135° F.). These results indicate that limited destruction of lipase in oxygen-free products may be expected at the low temperatures which may occur during concentration by vacuum evaporation. The inactivation of lipase should occur prior to such treatment, since some activation and lipolysis may result from the agitation incident to evaporation (6).

#### CONCLUSIONS

1. Dissolved copper causes no inactivation of lipase in normal whole milk in the absence of dissolved oxygen.
2. Normal milk lipase is susceptible to inactivation by dissolved oxygen, and this inactivation reaction is accelerated by heat and by dissolved copper.

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# THE "IN VITRO" CONVERSION OF INORGANIC NITROGEN TO PROTEIN BY MICROORGANISMS FROM THE COW'S RUMEN\*

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## INTRODUCTION

Recent work at this station (Hart *et al.* (1)) has shown that growing dairy calves are able to use inorganic nitrogen in the form of urea or  $\text{NH}_4\text{HCO}_3$  as a substitute for part of the protein in the ration. The most probable explanation given for this utilization is the production of protein from this nitrogen by the growth of bacteria in the rumen and later digestion of these bacterial cells in the fourth stomach and intestines. Species other than herbivora have failed to demonstrate an appreciable use of urea (2, 3, 4) due probably to the absence of the polygastric type of stomach. With this view in mind the following "in vitro" experiments were set up to test the above hypothesis of inorganic nitrogen utilization in ruminants via rumen bacteria.

## EXPERIMENTAL

Preliminary trials were inaugurated in which we attempted to duplicate the conditions found in the rumen. These experiments consisted in adding urea to rumen contents and following the fate of the inorganic nitrogen. All samples were incubated at 37° C. Results in this trial were negative since the level of inorganic nitrogen did not decrease.

The determination of ammonia nitrogen was made by placing an aliquot of the medium into a Kjeldahl flask to which 300 cc. of  $\text{H}_2\text{O}$  were added followed by 5 grams of  $\text{MgO}$ . The ammonia was then determined by distilling into standard acid. Foaming was prevented by adding liquid paraffin. The urea nitrogen was estimated by treating an aliquot of the medium, neutralized to methyl red, in a Kjeldahl flask with 10 cc. of a 1 per cent solution of urease which also had been neutralized to methyl red. This was incubated for 1 hour at room temperature and then treated as described above for the ammonia nitrogen. The inorganic nitrogen includes the urea and ammonia nitrogen.

In the next trial the animal's ration was used as the medium to which urea was added. This was inoculated with rumen liquid. The rumen liquid, which was obtained from a paunch fistula, was secured by expressing the

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rumen contents: Results were again negative. It was found that a short time after incubation the pH of the medium became acid (4.0-4.5). It has been shown at this station and by others (5) that the reaction of the cow's rumen ranges from a pH of 6.0 to 7.5. With this fact in mind experiments were started in an attempt to maintain an optimum pH. Using sodium phosphate buffer solutions (pH 7.5), 0.15 to 0.5 M, the pH could not be controlled for a sufficient length of time to permit a maximum bacterial growth.

When stronger phosphate buffer solutions were used (1 M) activity was depressed possibly due to the hypertonicity of the solution. However, with the 0.15 or 0.5 M buffers, during the period of optimum pH, disappearance of inorganic nitrogen was observed. The first indication of a conversion (decrease in  $\text{NH}_3\text{-N}$ ) was obtained on a synthetic medium. The medium consisted of 600 cc. of water, 1.1 gram of urea, 15 cc. of molasses, 100 cc. of 1 M  $\text{Na}_2\text{HPO}_4$ , and was inoculated with 20 cc. of rumen liquid.

The results obtained follow:

Hours of incubation	mg. $\text{NH}_3\text{-N}/100$ cc. medium	pH
0	74.8	7.50
24	68.0	7.35
48	63.0	6.40
72	49.5	4.80

An examination of the above data shows that the phosphate buffer was not able to maintain an optimum pH.

Further trials with  $\text{CaCO}_3$  as the buffer showed that it was possible to maintain the medium at a pH of 5.8 to 6.5. An excess of  $\text{CaCO}_3$  was always added, so that a part of it remained undissolved. Since this pH approximates the reaction of the rumen, in all subsequent trials  $\text{CaCO}_3$  was used as the buffer. In all these experiments uninoculated samples were also run as controls.

#### *Carbohydrate studies*

The carbohydrates used included corn molasses, cerelose (commercial glucose), starch and cellulose. The source of carbohydrate in the medium with the exception of cellulose all supported bacterial growth as measured by the  $\text{NH}_3\text{-N}$  disappearance. Cellulose failed as a source of energy for the organisms used. These experiments were all carried on in synthetic media.

Nutrient salts for bacteria were found to be essential in the synthetic medium containing cerelose or starch. The salt mixture had the following composition:

$\text{KH}_2\text{PO}_4$	—10	grams
$\text{K}_2\text{HPO}_4$	—10	grams
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	—4	grams
$\text{NaCl}$	—0.2	gram
$\text{MnSO}_4$	—0.2	gram
$\text{Fe SO}_4$	—0.2	gram

The mixture was dissolved and made up to a volume of 1 liter. Ten cc. of this solution per liter of medium were used when necessary.

The extent of disappearance of inorganic nitrogen, regardless of the source of carbohydrate (cellulose excepted), depended on the amount of carbohydrate in the media. The data secured follow:

Hours of incubation	mg. NH <sub>3</sub> -N/100 cc. medium	Sugar added
0	83.0	30 cc. corn molasses
24	74.5	0
48	50.0	0
72	46.0	20 cc. corn molasses
96	3.5	0
$\frac{83.0 - 3.5 \times 100}{83.0}$	95.7 per cent disappearance of NH <sub>3</sub> -N.	

As can be seen from the above table there was a large decrease in NH<sub>3</sub>-N up to 48 hours. At this point the rate of disappearance decreased. On the addition of more corn molasses the rate of disappearance again increased markedly.

#### Nitrogen studies

It was observed that 24 hours after inoculation of the media the amount of urea present was negligible. At the same time there was an increase in NH<sub>3</sub>-N that was comparable to the decrease in urea nitrogen. This seemed to indicate that there was an initial hydrolysis of urea to NH<sub>3</sub> followed by disappearance of the NH<sub>3</sub>-N. This led us to compare disappearance of NH<sub>3</sub> using NH<sub>4</sub>HCO<sub>3</sub> vs. urea as the inorganic nitrogen source. No difference in the rate of disappearance was observed.

Data illustrating the use of these two materials follow:

Hours of incubation	mg. NH <sub>3</sub> -N/100 cc. medium	
	NH <sub>4</sub> HCO <sub>3</sub> -N	urea-N
0	74	74
24	56	54
48	24	19
72	12	3

The question arises as to the fate of the inorganic nitrogen that disappeared in these fermentations. Was it lost from the media, or converted to protein? The first possibility was eliminated by finding that there was no loss of total nitrogen in the media; the Kjeldahl method was used to determine total nitrogen. At the same time there was a disappearance of NH<sub>3</sub>-N. Illustrative data follow:

Hours of incubation	mg. NH <sub>3</sub> -N/100 cc. medium	mg. total N/100 cc. medium
0	133	139
96	79	132
	difference $\frac{133 - 79}{133} = .54$	
		$\frac{132 - 139}{139} = .007$
		95 per cent of total N recovered.

The second possibility, namely that there was conversion of  $\text{NH}_3 - \text{N}$  to protein, was investigated. This involved the removal of the protein from the medium (bacterial cells) by filtering the medium through a filter cell. The filter cell used was made of finely powdered silica. The filter was prepared by forming a quarter inch pad of filter cell over a filter paper in a Büchner funnel. Ammonia nitrogen was run on one aliquot and the protein (filter cell) nitrogen determined by Kjeldahl on another aliquot. The following data were obtained:

Hours of incubation	mg. $\text{NH}_3 - \text{N}/100 \text{ cc. medium}$	Residual - N/100 cc. medium
0	60	2.9
72	42	19.2
difference	18 mg. $\text{NH}_3 - \text{N}$ lost	16.3 mg. residual N gained.
$\frac{16.3 \times 100}{18} = 90.6$ per cent recovery of $\text{NH}_3$ nitrogen as non-filterable N.		

The recovered non-filterable material was presumably bacterial cells which contained the  $\text{NH}_3 - \text{N}$  lost, which was also the residual nitrogen gained.

Poor conversion was observed in samples using the cow's ration as the medium to which  $\text{NH}_4\text{HCO}_3$  was added. The ration consisted of equal parts of corn and oats, timothy hay, and corn silage, and was added at a 15 per cent level. Since the greatest difference between the ration and the synthetic medium seemed to be the protein, the effect of varying the protein content of the medium was studied. The basal medium consisted of 350 cc. of water, 25 grams of cerelose, 20 grams  $\text{CaCO}_3$ , 50 cc. rumen inoculum, 3 cc. nutrient salts, 2.6 grams  $\text{NH}_4\text{HCO}_3$ , to which varying amounts of casein were added. The following results were secured:

Sample	Supplement	mg. $\text{NH}_3 - \text{N}$ remaining/100 cc. medium			
		0 hours	24 hours	48 hours	72 hours
1 .....	No casein	105	49	26	3
2 .....	2.5 gms. casein	104	88	74	53
3 .....	5.0 " "	105	91	93	96
4 .....	10.0 " "	101	93	98	100

The above data indicate that only on samples "no casein added" and "2.5 grams casein level" was there conversion; the other two levels of casein added to the medium were negative. A probable explanation is that the bacterial flora used protein nitrogen in preference to  $\text{NH}_3 - \text{N}$ , or that the bacterial proteolytic enzymes masked the drop in the  $\text{NH}_3 - \text{N}$  by forming  $\text{NH}_3$  from the protein as fast as the  $\text{NH}_3$  was built into bacterial cells.

Since the conversion over a 24-hour period represents merely an average of that period, hourly  $\text{NH}_3 - \text{N}$  determinations were made during the peak of activity of the bacterial growth in the media. This was done in order to

determine the maximum rate of conversion calculated on a 24-hour basis. The data secured follow:

Hours of incubation	mg. NH <sub>3</sub> -N/100 cc. medium	Conversion per 24 hrs./ 100 cc. medium
20	86	0.0 mg. N
22	85	12.0 " "
24	82	36.0 " "
26	79	36.0 " "
28	73	72.0 " "
30	66	84.0 " "
32	57	108.0 " "
34	50	84.0 " "

The maximum conversion calculated in this manner is much greater (108 mg. NH<sub>3</sub>-N per 24 hours per 100 cc. medium) than that found when determinations were made every 24 hours, which were never found to be over 50-60 mg. NH<sub>3</sub>-N/100 cc. medium, since they were an average of the 24-hour period. This suggests what could be expected in the rumen of the animal assuming optimum conditions existed in the paunch at all times.

The buffering action of cow's saliva is recognized as an important factor in maintaining the reaction of the rumen at a near neutral point. Since large amounts of starch are normally present in the ration the presence of amylase in the saliva could be readily invoked as a means of forming soluble sugars which would promote bacterial growth. The literature indicates that cow's saliva has no diastatic properties. Amylase activity of cow's saliva was determined on a starch medium adjusted to pH 6.8 and incubated at 37° C. The saliva was collected by having the cow chew on a sponge; the liquid in the sponge was expressed into a flask at short intervals. To 100 cc. of saliva that was saturated and covered with toluene, 10 cc. of a 3 per cent starch solution were added, and the reducing sugars determined with Fehling's solution by measuring at frequent intervals the Cu<sub>2</sub>O precipitated. The results indicated only a slight amyloytic activity.

Because of this slight amyloytic property the addition of cow's saliva to a synthetic starch medium inoculated with rumen liquid might enhance conversion due to an increase of available fermentable sugars for the bacteria. However, when cow's saliva was added to an inoculated starch medium, no increase in conversion was produced. Using maltose in place of starch the maltase activity of saliva was found to be negative. The possibility of diastatic activity in the rumen through bacterial action directly presents itself. Preliminary data on chemical changes in the rumen of the cow indicate a distinct diastatic action.

As evidence for this hypothesis, the diastatic action of rumen liquid was determined using the same technique as described above for saliva. In comparison to saliva, diastatic action of rumen juice is decidedly greater and

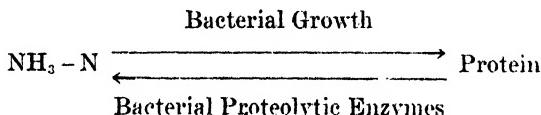
could very likely account for the hydrolysis which must occur in the breakdown of starch and possibly other polysaccharides.

Proteolytic activity of the saliva on a casein medium was also tested. Both tryptic and peptic enzymes were found to be absent. This finding indicated that there was no contamination of the cow's saliva we collected since considerable proteolysis occurs in the rumen.

#### DISCUSSION

The foregoing results have demonstrated that the conversion of inorganic nitrogen to protein may be obtained by inoculation of appropriate media with microorganisms contained in liquid from the cow's rumen. No attempts were made to inoculate the media with pure cultures of bacteria from the rumen since a multiplicity of different kinds undoubtedly exists in the paunch. For the same reason contamination was not considered as an important factor in this work.

In reviewing these results several criticisms appear obvious. This is especially true if an attempt is made to relate these findings to what actually happens in the rumen of the cow. In the rumen a maximum bacterial flora is always present while in "in vitro" experiments this flora must first develop. In this intervening time chemical changes such as proteolysis may be going on in the medium (using the ration as medium) which do not have time to occur in the rumen. This will lead to non-comparable results since two reactions are working in opposite directions.



All that is attempted in this work is to show how some of these factors influence the conversion. Through a fistula in the cow, studies on conversion in the rumen are now in progress. The food in the rumen is a continually moving mass, part of it being removed and new material being constantly added. This condition cannot be duplicated "in vitro." Further, the products of fermentation may have an adverse effect on conversion. In the rumen these products are continually being removed. From the above statements it also can be surmised why the results on a synthetic medium cannot be correlated closely with those obtained using the animal's ration as the medium.

However, the main fact obtains, namely, that given optimum conditions, which approach those in the rumen of the cow, conversion of inorganic nitrogen to protein can be demonstrated in "in vitro" experiments.

#### SUMMARY

1. Evidence is presented through "in vitro" experiments that conversion

of inorganic nitrogen to protein can occur through the use of bacteria from the cow's rumen.

2. Bacterial activity, and hence conversion, is dependent on the pH of the media, the optimum range being 5.5 to 7.

3. The carbohydrates used in the media were of equal efficiency in influencing conversion, with the exception of cellulose which was not an acceptable carbohydrate for these studies.

4.  $\text{NH}_4\text{HCO}_3$  is as efficient as urea in the rate of utilization by rumen organisms.

5. The decrease in  $\text{NH}_3$  can be accounted for by an increase in protein nitrogen.

6. The level of protein in the media has a negative influence on the decrease in  $\text{NH}_3 - \text{N}$ .

7. Amylolytic activity of rumen liquid has been demonstrated, with only slight activity in the saliva itself.

8. Proteolytic activity of cow's saliva is absent.

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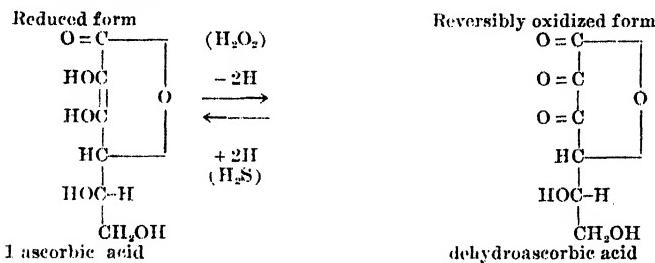


# THE EFFECT OF COMMERCIAL PRACTICES ON ASCORBIC ACID AND DEHYDROASCORBIC ACID (VITAMIN C) IN MILK<sup>\*1</sup>

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Ascorbic acid, analogous to vitamin C, exists in two chemical forms, reduced and reversibly oxidized, which possess equal biological activity for the prevention of scurvy (2). The reversibility of the relationship is indicated by the following equation:



The influence of processing methods on the stability of the two forms of vitamin C has not been clearly established. During the progress of this investigation Gjessing and Trout (1) reported on the stability of vitamin C in milk pasteurized at different temperatures and for varying intervals using the indophenol titrimetric technique to determine only the ascorbic acid.

The object of this study was to determine the influence of commercial practices on the two forms of vitamin C, ascorbic and dehydroascorbic acids. Little attention has been given to the practicability of producing a vitamin-C fortified milk on a commercial scale. Hence it was deemed advisable to study the production of such a product, for if it were available it should prove of significant value for general use in welfare work, in maternity wards, and in general malnutrition.

## EXPERIMENTAL

The literature contains numerous procedures for the determination of ascorbic acid in milk but only very few for the determination of the impor-

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<sup>1</sup> Presented before the Division of Agricultural and Food Chemistry at the 100th meeting of the American Chemical Society, Detroit, Michigan, Sept. 9-13, 1940.

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tant, equally biologically active dehydroascorbic acid. Kon and Watson (3) have suggested a procedure for the estimation of dehydroascorbic acid in milk but Woessner, Elvehjem and Schuette (4, 5) have shown that the use of a photoelectric colorimeter is essential for such a determination because it eliminates the interference due to other substances which are formed when the milk is treated with hydrogen sulfide. Their method which was used for this research is specific for ascorbic and dehydroascorbic acids.

The apparatus and reagents were identical with the photoelectric technique described by Mindlin and Butler (6) except that the potassium oxalate, cyanide, and metaphosphoric acid solutions were replaced by a modified Willberg (7) reagent (0.6 g.  $H_2C_2O_4 \cdot 2H_2O$ , 4.8 g. NaCl and 6.5 g.  $HPO_3$  in one liter of water) prepared accurately to insure proper pH in the colorimeter tube.

*Determination of ascorbic acid.* In the absence of strong light 25 ml. of milk are pipetted into a 125-ml. Erlenmeyer flask containing 75 ml. of modified Willberg reagent. The protein precipitate is removed by filtering through paper of quality similar to Whatman No. 42. Five ml. of the filtrate are measured into a colorimeter tube and 10 ml. of the indophenol solution are added. The contents are stirred and examined immediately. Since it is not always possible to obtain a filtrate that is crystal-clear, it is recommended that a small quantity of ascorbic acid be added after the original reading has been made, whereupon the correction due to the turbidity is determined. Thus, the true reading is equal to the original reading plus 100 minus the reading after the crystal of ascorbic acid has been added.

*Determination of dehydroascorbic acid.* After the addition of a few drops of dibutyl phthalate to the milk to prevent foaming, wet hydrogen sulfide is bubbled through the milk for exactly 20 minutes. Then as rapidly as manipulation will permit, 25 ml. of the hydrogen-sulfide-saturated milk are added to 75 ml. of modified Willberg reagent, and the whole is shaken well to break into small particles the curd which forms. The hydrogen sulfide is removed immediately by passing a vigorous stream of wet oxygen-free nitrogen through the mixture for 20 minutes. After the curd is removed by filtration, 5 ml. of the filtrate are measured into one of the colorimeter tubes. Then with the simultaneous start of a stop watch, 10 ml. of the dye acetate solution are added to the tube by means of a rapid delivering pipette, and the galvanometer readings at 15 and 30 seconds are recorded. The galvanometer reading corresponding to zero seconds can be considered equal to the difference of the galvanometer readings at 15 and 30 seconds subtracted from the galvanometer reading at 15 seconds.

Calculations are the same as those described by Mindlin and Butler (6); K under the conditions prescribed has a value of  $0.166 \pm 0.003$ .

*Milk supply and plant equipment.* The milk used for these studies was of the regular plant supply as received daily at the Department of Dairy

Industry of the University. It was handled by either one of two different procedures. In the manner of handling which shall be referred to later for convenience, as the A process, the milk was received at the intake in a stainless steel weighing tank and then dropped into a stainless steel holding tank. Next it was passed through a short piece of partially worn tinned-copper piping, a stainless steel positive-feed pump, an air-tight separator-clarifier and finally through a short piece of partially worn tinned copper piping into a vertical cylindrical spray-vat pasteurizer. In the B process, the milk was handled as in the A process until it was passed through the pump whence it was passed through a tubular preheater ( $90^{\circ}$  F.) and thence through the separator-clarifier. From the clarifier the milk was passed through forty feet of well worn tinned-copper piping into a stainless steel horizontal spray-vat pasteurizer.

#### THE INFLUENCE OF ELEVATED TEMPERATURES

The clarifier and tubular preheater used were found to exert no destructive or oxidative effect on ascorbic or dehydroascorbic acids that could in any way be considered significant.

In table 1 is summarized the effect of holder pasteurization on the ascorbic acid content of several normal milks and on the ascorbic acid content of milks containing added ascorbic acid. Lots I to V inclusive were handled by the A process and Lots VI and VII by the B process. Since the A process milks were to be homogenized they were pasteurized at a higher temperature ( $150^{\circ}$  F.) than the B process milks ( $145^{\circ}$  F.).

From the data (table 1) it is observed that the A process milks show an average destruction of ascorbic acid of 11.4 per cent and what may be considered complete destruction of dehydroascorbic acid. The overall destruction of the vitamin by this process is 20.2 per cent. This latter figure is in excellent agreement with values previously cited in the literature.

However, Kon and Watson (3) found that the loss in total ascorbic acid originally present in the milk is more than three times that suffered by the reduced form. Hence they claimed it is chiefly the reversibly oxidized form that is destroyed. The data (table 1) indicate that the loss in total ascorbic acid is never much greater than the loss of ascorbic acid itself and thus the results are not in agreement with those of Kon and Watson (3).

The quantity of dehydroascorbic acid remaining after pasteurization is less than the quantity present before pasteurization and is also less than the quantity of ascorbic acid lost during pasteurization. This indicates that part of the dehydroascorbic acid originally present in the milk which was formed from ascorbic acid by normal exposures of the milk to light, as well as that formed from the ascorbic acid during the pasteurization holding process undergoes a very rapid destruction. It appears that the dehydroascorbic acid is destroyed as rapidly as it is formed from the ascorbic

TABLE 1  
*The effect of vat pasteurization on the forms of ascorbic acid in milk*

Milk lot	Source of sample	Ascorbic acid per liter			Total per cent loss
		Reduced	Oxidized	Total	
I <sup>2</sup>	From vat before pasteurization	17.4	1.6	19.0	
	After pasteurization for 30 min. at 150° F.	15.9	0.0	14.9	21.5
II	From vat before pasteurization*	40.0	3.2	43.2	
	After pasteurization for 30 min. at 150° F.	38.4	1.1	42.0	2.7
III	From vat before pasteurization	14.4	6.1	20.5	
	After pasteurization for 30 min. at 150° F.	12.5	2.4	14.9	27.3
IV	From vat before pasteurization*	38.7	4.7	43.4	
	After pasteurization for 30 min. at 150° F.	33.0	0.7	33.7	22.3
V	From vat before pasteurization	17.1	2.8	18.9	
	After pasteurization for 30 min. at 150° F.	14.2	0.0	14.2	24.8
VI <sup>2</sup>	From vat before pasteurization	15.4	5.8	21.2	
	After pasteurization for 30 min. at 145° F.	10.5	4.9	15.4	12.6
VII	From vat before pasteurization*	39.9	10.3	50.2	
	After pasteurization for 30 min. at 145° F.	25.7	12.7	38.4	23.3

\* Fortified with added ascorbic acid.

<sup>1</sup> Lots I to V—Stainless steel vertical spray vat pasteurizer.

<sup>2</sup> Lots VI and VII—Stainless steel lined horizontal spray vat pasteurizer. Copper content 0.13 to 0.29 p.p.m.

acid. The limiting factor in destruction of total vitamin C, therefore, is not the rate of destruction of dehydroascorbic acid but rather the transformation of ascorbic acid to dehydroascorbic acid by heat. The reversibly oxidized form (dehydroascorbic acid) may be considered as being chiefly destroyed only if it is originally present in a larger quantity than the amount of ascorbic acid which is normally lost during the thirty-minute holding period. It is for this reason that statements in the literature which claim the flash pasteurization procedure to be less destructive than the holding process are believed to be sound. The destruction of the ascorbic acid appears to be more dependent on the time it is held at the elevated temperature than on the temperature itself; in other words, the rate of the conversion of ascorbic acid to dehydroascorbic acid has a small temperature coefficient whereas the decomposition of dehydroascorbic acid has a high temperature coefficient.

This conclusion is supported by the results obtained when handling milk by the A process including the experimental lots I to V (table 1). A somewhat different result was obtained when the milk was handled by the B method, including lots VI and VII (table 1). The processing of the milks in this instance involved considerably more copper piping which introduced

an average of 0.21 p.p.m. of copper into the milk. As a result dehydroascorbic acid was very rapidly formed from ascorbic acid and the percentage loss of ascorbic acid was as high as 35.6 per cent. The results of the measurement of dehydroascorbic acid shows that the copper present caused the formation of dehydroascorbic acid as rapidly as heat destroyed it. This can be more readily understood by comparing the total loss of ascorbic acid during the pasteurization with the quantity of dehydroascorbic acid originally present in the milk. The two figures are about equal. Copper, therefore, can be considered as accelerating the conversion of ascorbic to dehydroascorbic acid, a reaction ordinarily quite slow at elevated temperatures in the absence of light, so that it equals the rate at which dehydroascorbic acid is thermally destroyed. Thus an explanation is provided for the instability of ascorbic acid in the presence of copper in milk.

In the presence of copper the conversion of ascorbic to dehydroascorbic acid evidently has a high temperature coefficient. That the thermal decomposition of dehydroascorbic acid has a high temperature coefficient in the absence of copper is demonstrated by the fact that the dehydroascorbic acid produced by exposing copper-free milk to light can only be quantitatively recovered if the sample is kept cool; short heating to 100° C. will destroy completely the dehydroascorbic acid. Hence it is assumed that copper does not accelerate the irreversible oxidation of dehydroascorbic acid but only speeds the conversion of the heat-stable ascorbic acid to the heat-labile dehydroascorbic acid.

TABLE 2  
*The relative destruction of the forms of ascorbic acid in milk during rat pasteurization\**

Milk lot	Temperature at which sample was taken	Holding time	Ascorbic acid per liter		
			Reduced	Oxidized	Total
I**	80° F.	Minutes	17.1	2.8	18.9
	100° F.		16.0	...	...
	120° F.		16.0	...	...
	130° F.		16.2	2.0	18.2
	140° F.		15.8	...	...
	150° F.		16.0	1.1	17.1
II	150° F.	0      30	14.2	0.0	14.2
	140° F.		18.0	0.0	18.0
	150° F.		17.6	0.0	17.6
	150° F.		17.1	0.0	17.1
	150° F.		16.7	0.0	16.7
	150° F.		15.8	0.0	15.8

\* Vertical cylindrical stainless steel pasteurizer.

\*\* The time of heating from 80° F. to 150° F. was 16 minutes. Approximately 3 minutes elapsed between each temperature indicated.

In order that the manner in which the loss of ascorbic acid occurs in the vertical spray-vat pasteurizer (process A) might be still better understood measurements were made during the entire preheating and holding period of the milk while in the vat. The results of such an investigation are summarized in table 2. The data obtained were not influenced by the presence of copper in the milk.

During the preheating period a nine per cent loss in ascorbic acid was observed. It also appears that there was a general diminution of the dehydroascorbic acid so that by the time (16 minutes) the milk reached 150° F. there was little or no dehydroascorbic acid remaining. During the holding period the data denote there was a ten per cent loss of the ascorbic acid, the dehydroascorbic acid being destroyed as rapidly as it was formed. This observation confirms the reasoning which has already been presented.

Since nine per cent of the total destruction occurred during the short preheating period of 12 minutes, it is pertinent to note that most of the loss was due to the destruction of the dehydroascorbic acid already present in the raw milk. The loss during the thirty-minute holding period consisted wholly of ascorbic acid by way of the mechanism postulated. Thus it is reasonable to suppose that the superiority of the flash process over the holder method comes mainly from the fact that the milk is held at the higher temperature for a shorter period of time and not because of a rapid pre-heating period. The dehydroascorbic acid originally present in the milk no doubt is destroyed in the flash process but the ascorbic acid is probably little affected because of its low temperature coefficients coupled with the fact that it remains at the elevated temperature for such a short period of time.

From the foregoing discussion it can also be concluded that if a sample of milk is subjected to thermal treatment sufficient to cause a diminution of the ascorbic acid present there will also be a total destruction of the dehydroascorbic acid, including that present before the heat is applied and that formed during the time the milk is held at the elevated temperature.

#### THE EFFECT OF HOMOGENIZATION

The milk for these experiments was processed by the A method and was then passed from the pasteurization vat through stainless steel piping to the homogenizer. The homogenization was effected at 150° F. and 2000 lbs. pressure after which the milk was passed over a newly tinned copper cooler (40° F.). The cooler was protected from light with metal covers.

In table 3 are summarized the results of homogenizing 6 different lots of milk. Inspection of the data of both the normal and vitamin C fortified lots discloses that homogenization does not cause any destruction of ascorbic acid or formation of dehydroascorbic acid.

It also may be concluded from table 3 that passing milk over a tinned-copper cooler in good condition causes no loss of ascorbic acid or formation of dehydroascorbic acid.

TABLE 3  
*The effect of homogenization on the forms of ascorbic acid of milks<sup>1</sup>*

Sample taken	Ascorbic acid per liter					
	Reduced	Oxidized	Total	Reduced	Oxidized	Total
	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.
	<b>I</b>			<b>II<sup>2</sup></b>		
From vat before pasteurization .....	17.4	1.6	19.0	40.0	3.2	43.2
After 30 minutes at 150° F. ....	15.9	0.0	14.9	38.4	1.1	39.5
After homogenization <sup>3</sup> .....	14.5	0.0	13.6	39.5	0.0	39.5
After cooling .....	14.7	1.4	16.1	38.9	1.3	40.2
	<b>III<sup>2</sup></b>			<b>IV<sup>2</sup></b>		
After 30 mintues at 150° F. ....	35.7	2.5	38.2	33.0	0.7	33.7
After homogenization <sup>3</sup> .....	35.0	3.7	38.7	32.4	2.6	35.0
After cooling .....	35.7	1.3	37.0	33.0	0.0	32.6
	<b>V</b>			<b>VI</b>		
After 30 minutes at 150° F. ....	14.2	0.0	14.2	15.8	0.0	15.8
After homogenization <sup>3</sup> .....	12.8	2.3	15.1	15.8	0.0	15.8

<sup>1</sup> Vertical stainless steel pasteurizers and stainless steel homogenizer (2000 lb. pressure).

<sup>2</sup> Ascorbic acid added to milk before pasteurization.

<sup>3</sup> Sample taken while hot before the milk was passed over the cooler.

#### THE FEASIBILITY OF PRODUCING A VITAMIN C-FORTIFIED MILK

Reedman (8) and Kroker (9) suggested the feasibility of preparing a vitamin C-fortified milk. Kroker (9) suggested the addition of ascorbic acid to milk directly after it is flash-pasteurized and, because of the destructive effect that light has on the vitamin, he also suggested that the milk be marketed in dark glass bottles.

The fortification of milk handled by the A and B processes was studied. In the absence of significant quantities of copper these experiments which were conducted on a commercial scale (600 gallons) proved that such fortification is practical. The only limiting factors from the commercial standpoint are the present cost of ascorbic acid and the necessity of affording protection from light and exposed copper.

In series 1 (table 4) the milk was handled by the A process and divided equally into two identical vertical spray pasteurizer vats. The fortification with ascorbic acid was accomplished by dissolving the vitamin in 100 ml. of sterile copper-free water and pouring the solution slowly into the vat containing the milk; this operation was followed by a short counter flow mixing with a stirring rod to insure rapid distribution of the vitamin throughout

the milk. The addition of ascorbic acid had no froward effects upon the flavor of the milk.

TABLE 4  
*The stability of the forms of ascorbic acid in vitamin C fortified milk*

Sample taken	Ascorbic acid per liter					
	Unfortified			Fortified*		
	Reduced	Oxidized	Total	Reduced	Oxidized	Total
	mgs.	mgs.	mgs.	mgs.	mgs.	mgs.
Series I <sup>1</sup>						
From vat before pasteurization .....	17.4	1.6	19.0	40.0	3.2	43.2
After 30 minutes pasteurization at 150° F. ....	15.9	0.0	14.9	42.0	0.0	42.0
After 50 minutes pasteurization at 150° F. ....	14.7	0.5	15.2	38.4	1.1	39.5
After homogenization <sup>4</sup> .....	14.5	0.0	14.5	39.5	0.0	39.5
After cooling <sup>4</sup> .....	14.7	0.0	14.7	38.9	1.3	40.2
Series II <sup>2,3</sup>						
From vat before pasteurization .....	15.4	5.8	21.2	39.9	10.3	50.2
After 30 minutes pasteurization at 145° F. ....	10.5	4.9	15.4	25.7	12.2	38.4
After cooling .....	5.7	7.8	13.5	25.7	10.2	35.9
After 24 hrs. storage 40° F. ....	0.0	3.8	3.8	0.0	14.8	14.8

<sup>1</sup> A process.

<sup>2</sup> B process.

<sup>3</sup> 20 gms. ascorbic acid added to 730 quarts of milk in vat before pasteurization.

<sup>4</sup> Sample removed after being held for 30 minutes at elevated temperature.

<sup>5</sup> On analysis these samples contained 0.13 to 0.29 p.p.m. of copper.

The data (table 4) indicate that the losses of ascorbic acid in both the unfortified and the fortified milks upon pasteurization are normal. It may be seen, however, that a definite antiscorbutically better product can be produced at will. By adjusting the initial fortification, a milk could be easily produced that would be on a par antiscorbutically with human milk (50 to 70 mg. per liter). If the milk should become contaminated with copper from the pipe lines or bottling equipment, an attempted fortification would be impractical as the vitamin C would almost disappear in 24 hours.

Regulations permitting, a more economical fortification is possible if the vitamin is added at the close of the holding period. This fact is illustrated by the data of table 5. Consequently, the suggestion of Kroker (9) is the ideal method of fortification as flash pasteurization conserves the maximum amount of the natural vitamin already present in the milk.

#### THE EFFECT OF LIGHT

Kon and Watson (3), Houston, Kon and Thompson (10) and Hender-

TABLE 5  
*The stability of the forms of ascorbic acid added to milk before and after pasteurization\**

Sample taken	Ascorbic acid per liter		
	Reduced	Oxidized	Total
	mgs.	mgs.	mgs.
<b>I</b>			
From vat before pasteurization	14.4	6.1	20.5
After 30 minutes pasteurization at 150° F.	12.5	2.4	14.9
After 30 minutes pasteurization at 150° F., <i>ascorbic acid added</i> (16 gms. per 730 quarts)	35.7	2.5	38.2
After homogenization	35.0	3.7	38.7
After cooling	35.7	1.3	37.0
After 24 hr. storage in dark	29.0	3.4	32.6
<b>II</b>			
From vat before pasteurization	14.4	7.0	21.4
From vat before pasteurization, <i>ascorbic acid added</i> (16 gms. per 730 quarts)	38.7	4.7	43.4
After 30 minutes pasteurization at 150° F.	33.0	0.7	33.7
After homogenization	32.4	2.6	35.0
After cooling	33.0	0.0	32.6
After 24 hr. storage in dark	26.3	3.3	29.6

\* Vertical stainless steel spray pasteurizers and stainless steel homogenizer used (2000 # pressure).

son, Foord and Roadhouse(11) have studied the effect of light and the effect of different containers on the antiscorbutic activity of milk. The results of this investigation, as shown in table 6 confirm their qualitative observations that brown glass and wax-impregnated cartons protect the ascorbic acid from actinic rays of light considerably more than the ordinary clear glass milk bottle. While a difference in the actinic effect on the vitamin C was observed by using different bottles or containers, it should be noted that exposure of any of these bottles or containers to direct sunlight for sufficient period caused the formation of the labile dehydroascorbic acid. Milk in plain glass milk bottles which were carried on the regular delivery route (enclosed truck delivery) showed no losses in excess of those normally encountered when the milk is stored in the dark. This indicates that in the ordinary delivery of the milk serious exposure to light probably does not occur. The significant loss will occur, therefore, not while the milk is being processed (assuming no copper contamination) including preheating, pasteurization, homogenization and cooling but rather when the milk is exposed to sunshine after its delivery. The benefits of fortification would be nullified if subsequent protection of the milk from light is not provided.

#### THE EFFECT OF OTHER COMMERCIAL PRACTICES

The stability of the two forms of vitamin C in normal and vitamin

TABLE 6

*The value of containers in preventing losses of ascorbic acids in milk exposed to light<sup>1</sup>*

	Ascorbic acid per liter		
	Reduced	Oxidized	Total
	<i>mgs.</i>	<i>mgs.</i>	<i>mgs.</i>
<b>I. Analyzed immediately:</b>			
<i>Unfortified Milk</i>			
Unexposed .....	17.0	0.0	17.0
Clear glass bottle, exposed to light 1 hr. ....	12.1	3.9	16.0
Brown glass bottle, exposed to light 1 hr. ....	17.0	0.0	17.0
Wax container, exposed to light 1 hr. ....	17.0	0.0	17.0
<i>Fortified (Added C) Milk</i>			
Unexposed .....	40.6	0.0	40.6
Clear glass bottle, exposed to light 1 hr. ....	32.0	7.1	39.1
Brown glass bottle, exposed to light 1 hr. ....	40.4	0.0	40.4
Wax container, exposed to light 1 hr. ....	39.8	0.6	40.4
<b>II. Exposed and then stored in dark 8 hrs. at 40° F. before analysis</b>			
Unfortified, clear glass bottle, exposed to light 1 hour .....	5.8	3.1	9.9
C-fortified, clear glass bottle, exposed to light 1 hour .....	23.0	5.0	28.6
C-fortified, brown glass bottle, exposed to light 1 hour .....	28.6	4.9	33.5

<sup>1</sup> A Process—homogenized milks. Overcast but bright day; no direct sunlight. Quart containers were used.

C-fortified milk processed by various methods was not in any way altered when further fortified by the addition of vitamin D concentrates processed in stainless steel equipment.

The use of sodium metaphosphate to lower the curd tension as recommended by Schwartz, Jones, Mack and Vance (12) was found neither to accelerate the oxidation of ascorbic acid or protect it from oxidative catalysis of copper (0.15 p.p.m.). Identical results were obtained when a pancreatic enzyme concentrate (Armour and Co.) was used (1 part in 25,000). However, it is pertinent to note that although the enzyme concentrate lowered the curd tension in addition to preventing copper-induced oxidized flavor it did not prevent the rapid oxidation of ascorbic acid. Thus it appears that ascorbic acid is not related to copper-induced oxidized flavor in milk.

#### CONCLUSIONS AND SUMMARY

Pasteurization by the holder methods was found to cause a 20 per cent loss of total ascorbic acid. After pasteurization no dehydroascorbic acid was found in the milk. The advantage of the flash pasteurization process in preserving the vitamin C content of milk seems dependent upon the fact that the destruction of the ascorbic acid appears to be more dependent on the time that it is held at the elevated temperature than on the temperature

itself; in other words, the rate of the conversion of ascorbic acid to dehydroascorbic acid has a small temperature coefficient whereas the decomposition of dehydroascorbic acid has a high temperature coefficient.

The most serious losses in antiscorbutic activity of milk during its processing and delivery were caused by contamination by copper and exposure to light. It is practical to produce a vitamin C-fortified milk on a commercial scale but the rigid exclusion of copper and protection from light are essential if the fortified milk is to be marketed through normal channels.

Tubular preheating (90° F.), clarification, homogenization, cooling and protected delivery individually, or collectively, were found to cause no loss of ascorbic acid or dehydroascorbic acid in milk. Likewise, no loss was caused by the addition of vitamin D concentrate followed by homogenization. Use of sodium metaphosphate or pancreatic enzyme were found to have neither a protective or detrimental effect on the vitamin C. The enzyme concentrate prevented copper-induced oxidized flavor but did not inhibit the usual rapid disappearance of ascorbic acids.

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## THERMODURIC BACTERIA IN PASTEURIZED MILK. A REVIEW OF LITERATURE

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During recent years there has been a growing interest in the study of thermoduric bacteria in pasteurized dairy products. Among the factors which have been operative in stimulating this interest, the following four have been important.

1. Increasing emphasis on the part of certain Departments of Health on low bacteria counts.

2. The distinctly higher counts obtained on agar plates when the medium is enriched, as by the change from the old standard nutrient agar to the tryptone glucose extract milk agar adopted as official in the 1939 edition of Standard Methods for the Examination of Dairy Products (1). It was pointed out by Sherman in 1916 (2) that as simple a change in the old standard nutrient agar as the addition of lactose would give much higher counts. Since the adoption of the tryptone glucose extract milk agar as standard, data has been accumulating showing that higher counts are obtained with the new as compared with the old medium (3). In addition, numerous articles were published previously showing the increases in count obtained on other enriched media similar to, but differing slightly in composition from the one finally adopted as standard (4, 5, 6, 7, 8).

3. The much higher counts obtained when the temperature of incubation is lowered. When the American Public Health Association was considering the change in the composition of the agar, referred to above, it was also considering a reduction from 37° C. to 32° C. (98.6° F. to 89.6° F.) in the temperature of incubation. Studies made and published in connection with this proposal showed that counts on all dairy products except dry milk are increased by such a reduction in the incubation temperature (3, 5, 6, 9, 10).

4. The rapid development in recent years of equipment for pasteurizing milk by the high-temperature, short-hold method. This method has decided advantages over the low-temperature, long-hold method from the standpoint of engineering and cost efficiency (11, 12, 13). Moreover, it is safe from the standpoint of destruction of pathogenic bacteria (11, 13, 14, 15, 17); phosphatase is destroyed (13, 16); flavor is not affected (13, 16); and cream volume is not reduced (13, 14, 16). However, bacteria counts are apt to be higher unless thermoduric organisms are eliminated from the supply, a point that will be discussed more in detail later in this review.

### A. BACTERIA SURVIVING LOW-TEMPERATURE PASTEURIZATION

When pasteurization first came into general use, it was assumed, especially by the medical bacteriologists, that most, if not all, of the organisms surviving would be spore-formers (18, 19), although Russell and Hastings (20) had reported in 1901 the discovery of a micrococcus which could survive heating to 76° C. (168.8° F.).

The classical studies on the bacteriology of pasteurized milk by Ayers and Johnson (21, 22, 23, 24, 25) in 1913-1916 established several important facts, as listed below.

1. The relative proportions of acid-producing, inert, alkali-producing and peptonizing bacteria are about the same in milk pasteurized at 145° F. (62.8° C.) for 30 minutes as in raw milk (21, 22, 25).
2. In milk pasteurized at 145° F. (62.8° C.) for 30 minutes (22), the percentage of each class of organism was as given in table 1.

TABLE 1  
*Organisms surviving pasteurization at 145° F. for 30 min.*

Class of organism	Grade A	Grade B
Acid without coagulation	61.87	34.82
Acid with coagulation	17.91	31.89
Inert	9.06	24.00
Alkali producing	9.77	5.63
Peptonizing	1.39	3.59
Total acid producers	79.78	66.71

3. As the temperature of pasteurization increases (with 30 minute holding), the proportion (but not the total number) of acid-producers and alkali-producers decreases, the proportion of inert organisms increases somewhat, and the proportion of peptonizers increases markedly (21, 22, 25).

4. Of a group of 139 cultures of acid-producers which were studied, 127, or 96.20 per cent, were cocci.
5. Of 43 alkali-producers, 31, or 72.09 per cent, were cocci.
6. Of 50 peptonizers, 34, or 68 per cent, were cocci.
7. The members of the inert group were not classified morphologically.
8. Of the three groups of acid-producers, alkali-producers and peptonizers, comprising 232 cultures, 192, or 82.71 per cent, were cocci (22).
9. Heating at 145° F. (62.8° C.) for 30 minutes in milk had the effect shown in table 2 on 139 cultures of streptococci isolated from four sources (23).

TABLE 2

*The effects of heating at 145° F. for 30 minutes on 139 cultures of streptococci from 4 different sources*

Source	Number of cultures tested	Number of cultures surviving	Per cent of cultures surviving
Feces	45	9	20.00
Udder	40	7	17.50
Mouth	36	13	36.11
Milk	18	17	94.44
Total	139	46	33.07

10. Colon bacilli are largely killed by heating to 145° F. (62.8° C.) for 30 minutes (24).

11. Bacteria do not multiply faster in pasteurized milk than they do in raw milk (21).

Most of the studies made by Ayers and Johnson were on milk of relatively high bacteria count. More recently three studies of the effect of pasteurization on the flora of low-count milk have been published (26, 27, 28), and the results differ from those of Ayers and Johnson. Instead of the percentage of acid-producing, inert, alkali-producing and peptonizing bacteria remaining unchanged by the pasteurization, as reported by Ayers and Johnson, the more recent work showed that the percentage of acid-producers was reduced when low-count milk was pasteurized, so that when spoilage occurred it did not consist of a typical acid coagulation, but rather a development of off-flavors (26), rennet coagulation at low acidities (27), and other similar types of spoilage (28). None of these three investigations showed peptonizing bacteria over-growing other types in this low-count pasteurized milk. However, it is obvious that the surviving flora and the consequent type of spoilage (when spoilage occurs) are far more desirable in the higher-count milks studied by Ayers and Johnson than in the lower-count milks studied by these more recent workers. Such an unfavorable change in the type of the surviving flora and in the type of spoilage may generally be expected where counts are forced down to the point where but few organisms, aside from the udder flora, get into the milk.

In 1923 Robertson (29) reported the presence on agar plates made from pasteurized milk of many punctiform colonies, most of which were micrococci, with a few rods and streptococci. Most of these organisms failed to curdle milk.

Robertson, Yale and Breed (30) reported in 1926 finding, on microscopic examination of pasteurized milk from certain plants, large numbers of spore-forming rods, belonging to nine species, as listed in table 3.

TABLE 3  
*Spore forming bacilli isolated from pasteurized milk*

Name	Number of cultures	Per cent
<i>B. subtilis</i>	48	34.2
<i>B. mesentericus</i>	29	20.7
<i>B. vulgatus</i>	22	15.7
<i>B. circulans</i>	21	12.0
<i>B. albolactis</i>	10	7.2
<i>B. lacterosporus</i>	2	1.5
<i>B. panis</i>	1	0.7
<i>B. cereus</i>	1	0.7
<i>B. mycoides</i>	1	0.7
Not identified	5	3.6
Total cultures	140	100.0

Most of them had been killed by the pasteurization, so that plate counts were not high. The source of the spores was found to be "milk stone" on the equipment, due to improper cleaning.

Robertson (31) in 1927 isolated a group of thermoduric organisms by holding raw milk samples at pasteurizing temperatures and making plates at hourly intervals up to seven hours. Cultures were also obtained from plates sent to him from pasteurizing plants in various cities. Only those cultures showing 90 per cent survival on being pasteurized in milk were studied. The species found to meet these conditions, in the order of their frequency were: *Sarcina lutea*, *Streptococcus thermophilus*, *Microbacterium lacticum*, *Micrococcus conglomeratus* and *Sarcina rosea*. The decreasing order of thermal resistance of these cultures was found to be: *Microbacterium lacticum*, *Sarcina lutea*, *Streptococcus thermophilus*, *Sarcina rosea* and *Micrococcus conglomeratus*.

In 1927 Fay (32) studied certain thermoduric organisms (55 cultures) surviving thirty-minute pasteurization at 143° F. (61.6° C.). Sugar was necessary for their growth on agar, and they would grow on standard agar only if the dilution was not greater than 1:100. Most of the cultures were very short rods (*Streptococcus lactis* type) or cocci growing in short chains, up to 10 cells long. Only about 10 per cent were long rods.

Brannon and Prucha (33) in 1927 submitted two-to-five-hour-old cultures of 47 unidentified non-spore-forming organisms to a temperature of 144.5° F. (62.5° C.) for 35 minutes in milk and found two surviving. Of these, one was not examined further, and the other was a micrococcus. In another series of cultures, the identity of which was known, treated in the same way, *Sarcina lutea* and the following four spore-formers survived: *B. subtilis*, *B. ramosus*, *B. butyricus* and *B. glaligu*.

Hammer and Trout (34) reported in 1928 that yellow micrococci commonly survive pasteurization. These organisms produced only slowly any change they brought about in milk.

Hucker (35) in 1928 studied 180 strains of cocci surviving pasteurization. Of these, 76, or 42.2 per cent, were micrococci, the most frequently occurring species of which were: *M. epidermidis*, *M. candidus*, *M. varians* and *M. luteus*. Of the streptococci, *S. thermophilus* was more common than any other species of cocci. Other lactic acid streptococci found were *S. faecium* and *S. liquifaciens*. *Streptococcus lactis* did not survive pasteurization temperatures. Hucker found that holding raw milk at 50° F. (10° C.) as compared with 68° F. and 86° F. (20° C. and 30° C.) for four hours prior to pasteurization did not greatly affect the total number of surviving cocci, but that the higher temperatures of pre-holding (68° F. and 86° F.) caused the percentage of *S. thermophilus* in the pasteurized milk to increase at the expense of the other species of cocci. Pasteurization was at 142° F. (61.1° C.) for 30 minutes.

Pricket (36) in 1929 studied 480 cultures of thermoduric bacteria forming "pin point" colonies, isolated from raw and pasteurized milk, from materials collected from farms producing milk that contained thermoduric

bacteria, from milk powders, media that had been sterilized in the autoclave, and from pea-blanche liquor. Seven types of organisms were observed as follows: Spore-forming rods, Non-spore-forming rods, Streptococci, Micrococci, Sarcinae, Actinomyces and Yeast.

Of these, only the spore-forming rods and certain Actinomyces were thermophilic.

The thermoduric micrococci were tentatively identified as: *M. candidus*, *M. epidermidis*, *M. luteus* and *M. albus*.

The streptococci were: *S. thermophilus*, *S. glycerinaceus* and *S. liquefaciens*.

The thermophilic spore-formers were: *B. subtilis*, *B. terminalis* var. *thermophilus*, *B. michaelisii*, *B. calidus*, *B. thermoalimentophilus*, *B. aerothermophilus*, *B. thermoliquefaciens*, *B. nondiastaticus*, *B. caladolactis* and *B. kaustophilus*.

Examination of a collection of cultures labelled *B. subtilis* showed two types. The *B. subtilis* Cohn type as described by Ford grew luxuriantly at 122° F. (50° C.) but the other type, resembling *B. cereus* Frankland did not grow at that temperature.

The name *B. kaustophilus* was proposed for a new species isolated.

Sherman and Pauline Stark (37) studied, in 1931, 294 cultures of streptococci from milk and other sources which grow actively at 113° F. (45° C.). All survived heating for 30 minutes in milk at 145° F. (62.8° C.). The most prevalent types were: *S. thermophilus*, *S. bovis*, *S. inulinaceus*, *S. fecalis*, *S. glycerinaceus*, *S. liquefaciens* and *S. zymogenes*. The authors raise the question as to whether *S. inulinaceus*, *S. glycerinaceus* and *S. zymogenes* should be considered as varieties of *S. bovis*, *S. fecalis* and *S. liquefaciens*, rather than as separate species.

McRady and Langevin (38) state that organisms of the coli-aerogenes group are seldom found in 1 cc. of properly pasteurized milk.

Minett and Pullinger (39) examined 49 samples of commercially pasteurized milk from six plants in England for the presence of *S. agalactiae*, the organism associated with the commonest form of bovine mastitis. Although this organism is very common in raw milk, they could demonstrate it in only one of their 49 samples of pasteurized milk.

Sherman (40) reported in 1936 that nine per cent of the samples of pasteurized milk examined contained hemolytic streptococci, but that the maximum number per cubic centimeter was 50, and that no pathogenic organisms could be found.

Macy (41) in 1939 reported on a study of high bacteria counts in pasteurized milk. He stated that the bactericidal efficiency of pasteurization is greater in summer than in winter. He studied 81 cultures isolated from 37° C. standard agar plates made from pasteurized milk, and classified them as shown in table 4. He found that dirty farm utensils were the usual sources of thermoduric bacteria.

TABLE 4  
*Reactions in litmus milk of cultures isolated from agar plates of pasteurized milk*

Type of Organism	Cultures			Caseolytic			Acid, no coagulation			Acid, coagulation		
	Number	Percent of total	Number	Percent of group	Number	Percent of group	Number	Percent of group	Number	Percent of group	Number	Percent of group
Spore-forming rods	13	16.05	13	100.00	7	53.84	9	0	9	0	1	0
Short, non-spore-forming rods	23	28.39	0?	0?	0?	0?	0	0	0	0	0	0
Streptococci	19	23.45	0?	0?	8.33	19	5.26	18	5.26	18	94.74	0
Micrococci	24	29.63	2	1	8.33	19	79.17	0	0	0	0	0
Sarcinae	2	2.47	0*	0?	0?	1	50.00	0	0	0	0	0
Total	81	100.00	15	18.51	28	34.57	18	34.57	18	34.57	18	22.22

Taylor (42) in 1924 was apparently the first one to use laboratory pasteurization of the milk from individual farms in controlling high counts in commercially pasteurized milk. He reported that improperly sterilized "milk contact surfaces" on the farm or in the milk plant were common sources of the thermoduric organisms, and that some farms are apt to give trouble continually.

Hussong and Hammer (43) in 1931 pasteurized morning's milk from individual farms in test-tubes at 142° F. (61.1° C.) making plate counts both before and after pasteurization. Large variations occurred in the percentage of organisms killed, both in samples from different farms and in different samples from the same farms. In the case of one farm having high counts in the pasteurized milk, a change in methods of caring for utensils and equipment on the farm resulted in lower initial counts and higher pasteurization efficiencies, and when initial counts increased again, due to hot weather, the higher efficiencies persisted.

A group of workers from United Dairies, London, England (Anderson and Meanwell, 1931; Davies, 1931; and Meanwell, 1939) (44, 45, 46) have published articles from which the following conclusions may be drawn.

1. Standard agar enriched with 0.5 per cent sterile milk is a satisfactory plating medium for the control of pasteurized milk.
2. There is a great variation in the number of heat-resistant organisms present in raw milk from different sources.
3. Under ordinary farm conditions and without utensil sterilization, more heat-resistant organisms are present in machine-produced milk than in hand-produced milk.
4. There is no constant relationship between the number of organisms present in raw milk and in the same milk after pasteurization.
5. Non-cooling of milk at the farm frequently encourages the development of heat-resistant organisms.
6. Heat-resistant organisms frequently originate from the surfaces of unsterilized utensils.
7. Daily sterilization of milking vessels resulted in nearly eliminating the heat-resistant organisms from the milk.
8. A simple quantitative plate count of raw milk affords little information as to the suitability of the milk for pasteurization.

It should be pointed out that this English group was the first to use laboratory pasteurization of the milk from individual farms, with a plate count of the pasteurized milk, as a routine method on a large scale in the control of high counts in pasteurized milk, although Taylor (42) and Hussong and Hammer (43) had both done it on a smaller scale prior to that time. Despite the good results reported, the method was ignored by dairy bacteriologists in this country until after the advent of high-temperature pasteurization and

the adoption of milk agar, both of which tend to show up the presence of thermoduric bacteria, forced the industry to adopt the method. The findings of these workers that thermoduric bacteria are associated with improperly cared-for farm utensils has been confirmed, as will be seen later in this review. The use of milk agar by the English group, several years before its official adoption in this country, was probably partly responsible for their recognition of the importance of the problem of the thermoduric bacteria.

The findings reported by various investigators (41, 42, 43, 44, 45, 46) that dirty farm utensils are the principal source of thermoduric bacteria is not surprising, since it has been shown that stable air (47) and unsanitary stables in general (48) have little effect in increasing the original bacterial contamination of milk, while utensils (49) and especially milking machines (50) supply most of this original contamination.

Moreover, at least one report has been published showing that thermoduric organisms grow readily in non-cooled milk (44).

#### B. BACTERIA SURVIVING HIGH-TEMPERATURE, SHORT-HOLD PASTEURIZATION

It seems to be generally conceded by investigators who have published work on high-temperature, short-hold pasteurization that bacteria counts are higher than in milk pasteurized by the low-temperature, long-hold method (11, 13, 51, 52, 53, 54, 55, 56, 57). However, some of these reports give no data to show the extent of the difference in count (11, 13, 52, 55, 57). Dotterer (51) presents considerable data showing that the high-temperature, short-hold method gives materially higher counts than does the low-temperature, long-hold method. He used single lots of milk divided into two portions, which were pasteurized by the two different methods. Parfitt (53) gives some data that seems to indicate that the high-temperature short-hold method gives, in certain plants, *lower* counts than does the other method, but his data must be discounted somewhat because it appears that his comparisons were made, not on single lots of milk divided into two parts and pasteurized by the two methods, but on different lots of milk, although the statement is made that the milk did all come from "a common source". Moreover, Parfitt intimates that the high-temperature short-hold method of pasteurization gives the higher counts, since he says in his conclusions "that a closer control of thermoduric organisms is necessary for low bacterial counts in milk pasteurized by the high-temperature short-hold method."

Quin and Burgwald (54) state that laboratory pasteurization shows little difference in the bactericidal results of holding 15 seconds at 160–162° F. (71.1°–72.2° C.) or 30 minutes at 143° F. (61.7° C.), but that commercial pasteurization gave an average count of 50,271 for high-temperature and 35,087 for low-temperature pasteurization. Yale (56) found that 18 lots of milk pasteurized in commercial apparatus at 143° F. (61.7° C.) for 30 minutes gave an average count of 17,200, whereas the same lots of milk pasteur-

ized in commercial apparatus at 160° F. (71.1° C.) for 15 seconds gave a count of 20,600. The same comparison for laboratory pasteurization was 6,490 and 12,200 per cc.

As in low-temperature, long-hold pasteurization, there seems to be little relationship between counts before and after pasteurization by the high-temperature, short-hold method (53).

Laboratory pasteurization tends to give lower counts than does pasteurization in commercial apparatus, regardless of the temperature and time used (53, 54, 56).

Several of the investigators who have published reports on high-temperature short-hold pasteurization have adopted the method of controlling high counts in the finished product that was first suggested by Taylor (42) and was extensively used by the United Dairies group in London, England (44, 45, 46). Samples of milk from individual farms are pasteurized in the laboratory and plate counts made. Those farms sending in milk the count of which is high after pasteurization are visited by an inspector, who attempts to find and correct the cause of the high count. There is uniform agreement among reports of such work that the thermoduric organisms originate principally in dirty farm utensils, and that cleaning up these utensils results in reduction of the count (11, 42, 43, 44, 45, 46, 51, 53, 55). Milking machines seem to be especially fertile sources of the offending organisms (11, 53, 55, 57, 58). Parfitt (53) shows a log average count of 630 for fifty hand-milking farms, 8,500 for fifty machine-milking farms. Prucha and Parfitt (57) presented the data in table 5 showing the relationship between the number of thermoduric organisms per cubic centimeter in milk from individual farms and the use of milking machines.

TABLE 5

*Relationship between the number of thermoduric organisms in milk and the use of milking machines*

Thermoduric count per cc.	Number of farms with milking machines	Number of farms without milking machines
5,000 .....	0	20
10,000 .....	0	7
20,000 .....	38	1
30,000 .....	30	0
40,000 .....	21	0
50,000 .....	16	0
Over 50,000 .....	14	0
Total .....	119	28

Cans may also be a serious source of these thermoduric organisms (58). Krueger, of the Chicago Department of Health (11), recommends that milking machine tubes be stored, when not in use, in 0.5 per cent sodium hydroxide solution, that they be boiled in such a solution occasionally, that

all milk stone be kept off of all farm equipment, and that routine cleaning practices on the farm be good, if thermoduric organisms are to be held in check.

The technique of laboratory pasteurization of large numbers of samples as a control procedure seems to have been very generally the low-temperature long-hold method. The English group used that method (44, 45, 46), as would be expected, since they were also using it in their plant operations. Parfitt (53) used 144° F. (62.2° C.) for 30 minutes and his results in controlling high counts in commercial high-temperature pasteurization were apparently satisfactory. Theoretically, it might be better to use the high-temperature method for laboratory work in controlling a commercial high-temperature operation. Two German workers have described a laboratory high-temperature pasteurizer (59), but it is extremely complicated. Quin and Burgwald (54) also used such an apparatus, but it would be difficult to clean and also difficult to use for routine pasteurization of large numbers of small samples. A satisfactory method has supposedly been developed in this country (60). The difficulty in laboratory high temperature short-hold pasteurization lies in obtaining the very rapid heating and cooling necessary to be strictly comparable with the commercial equipment.

The claim has been made that clarification of the milk after the regenerative stage of heating, with the milk at a temperature of about 125° F. (51.7° C.), will materially reduce the count (51, 52), but this has been denied by other workers (53, 55).

There seems to have been very little work done on the identity of the organisms surviving high-temperature short-hold pasteurization. One German worker (61) reported that *E. coli* survived a high-temperature pasteurization, and that the difficulty was eliminated when the low-temperature long-hold system was installed. However, no details of temperature and time are given, and the date of the experiments was 1924-25, so that it is probable no sufficiently accurate controls of time and temperature of heating were available for commercial equipment. No recent work on the incidence of *E. coli* in milk pasteurized commercially by the high-temperature short-hold method appears to have been published, but in view of the emphasis placed on the absence of this organism from pasteurized milk by public health authorities in this country, the mere fact that survival of the organism is not mentioned in the literature is fairly conclusive evidence that its presence in milk pasteurized by this high-temperature method is not a serious problem.

The only other work published on the survival of a definite group of organisms in milk pasteurized by the high-temperature short-hold method is the observation of Eglinton and Yale (58) that yellow, heat-resistant micrococci, which they state are common in milking machines and milk cans, often appear on agar plates made from pasteurized milk, especially where the high-temperature method of pasteurization is used.

## C. THE BACTERIAL FLORA OF DAIRY UTENSILS

Inasmuch as the principal source of thermoduric bacteria seems to be farm utensils, it appears that any literature available on the bacterial flora of these utensils should be of interest in this connection.

In 1924 Whiting (62) studied 357 cultures isolated from milk cans. The distribution of these cultures is shown in table 6.

TABLE 6  
*The distribution of cultures isolated from milk cans*

Type of organism	Number of cultures	Per cent of cultures
None-spore-forming rods	216	60.5
Micrococci	105	29.4
Sporeforming rods	36	10.1
Total	357	100.0

The species of micrococci present, in the probable order of their abundance, were: *M. aureus*, *M. conglomeratus*, *M. varians*, *M. luteus*, *M. flavus* and *M. cinereus*.

The first three are heat-resistant (31, 35, 36).

In the same year Robertson (63) studied 721 cultures from milking machines. He found that, when brine-hypoehlorite solutions were used for sterilizing the machines, the white, Gram-positive cocci were the commonest organisms. The Gram-negative rods and *Streptococcus lactis* were quite common under all conditions, but they formed a larger proportion of the total flora as the condition of the machines became less sanitary. The alkali-forming rods appeared to be associated with a treatment in which the tubes were submerged in cold water or in old sterilizing solutions of inadequate strength. A few cultures of the colon-aerogenes group were isolated. Sporeformers were rare. Molds (primarily *Oidium lactis*) and yeasts were found in accumulations of old milk in the tubes, stanchion hose and moisture traps. Actinomyces were found in small numbers in machines well-sterilized with hot water, and were regarded as dust contamination.

Of the 721 cultures isolated, 265, or 36.7 per cent, were micrococci. Of these, 78 or 10.8 per cent of the total of 721 were white heat-resistant micrococci, and 54 or 7.5 per cent were yellow heat-resistant forms.

The next year Robertson studied in detail these 265 cultures of micrococci (64). They seem to be able to survive sterilization with sodium chloride brine or with sodium or calcium hypochlorite, or chloramines, and therefore they are the commonest organisms present when these sterilizing compounds are used. Eleven species were identified. In the order of their probable abundance they are: *M. candidus*, *M. freudenreichii*, *M. casei*, *M. conglomeratus*, *M. epidermidis*, *M. varians*, *M. flavus*, *M. aurantiacus*, *M. luteus*, *M. albus* and *M. aureus*.

The first four species were sufficiently common to be regarded as a part of the normal bacterial flora of the milking machines. The other species are probably somewhat accidental contaminants.

After nine months without transferring, an attempt was made to revivify them. The attempt was successful with 49 cultures, and the species most common among these 49 were: *M. conglomeratus*, *M. casei* and *M. freudenreichii*. Apparently these three species were able to withstand drying better than *M. candidus* and other species.

As has been already shown, six of these species of micrococci are able to survive low-temperature pasteurization (see references 31, 35, 36).

Eglinton and Yale, in a paper previously referred to (58), found that the yellow, heat-resistant micrococci common on agar plates made from pasteurized milk, especially where the high-temperature method of pasteurization was used, were found to originate in milking machines and to a lesser extent in milk cans. While he did not identify any cultures, the species of micrococci producing yellow pigment (65) are: *M. conglomeratus*, *M. citreus*, *M. flavus*, *M. variens* and *M. luteus*.

It has already been noted that all of these but *M. citreus* and *M. flavus* have been shown to be heat-resistant (31, 35, 36). *M. conglomeratus* is apparently one of the most common organisms in both milking machines and milk cans (62, 64). It is heat-resistant (31).

The question now arises: Where do these heat-resistant micrococci come from? The fact that they are apparently able to resist heat and also killing by sodium chloride brines and by chlorine sterilizers acts as a means of selective enrichment, since they readily withstand the commonest methods of sterilizing farm utensils. But how are they introduced into the utensils? The answer is readily available in three studies on the udder flora of cows. In 1913 Harding and Wilson (66) studied the udder flora of cows. In 900 samples of aseptically drawn milk, they found 71 groups of organisms, none of which were sporeformers. About 75 per cent were micrococci. Fifteen years later, Alice Breed (67) studied the micrococci present in the normal cow's udder. The species she found, as well as her classification of the species isolated by Harding and Wilson, are listed in table 7.

It is especially significant that Robertson (31), Hucker (35) and Pricket (36) have found six species of micrococci to be heat-resistant. These species are listed as follows with the reference showing them to be heat-resistant: *M. albus* (36), *M. candidus* (35, 36), *M. conglomeratus* (31), *M. epidermidis* (35, 36), *M. luteus* (35, 36) and *M. varians* (35).

The studies of Harding and Wilson (66) and of Alice Breed (67), just described, show that these heat-resistant micrococci comprise better than 40 per cent of the micrococci present in the udder.

Evans (68) in 1916 studied the bacteria found in milk freshly drawn from normal udders, and found micrococci in 58.8 percent of 192 samples

TABLE 7

*Classification of the micrococci present in the normal cow's udder*

Species	Breed		Harding & Wilson		Combined	
	Number	Per cent	Number	Per cent	Number	Per cent
<i>M. aureus</i>	33	18.8	6	12.0	39	17.2
<i>M. aurantiacus</i>	24	13.6	1	2.0	25	11.1
<i>M. freudenreichii</i>	23	13.1	2	4.0	25	11.1
* <i>M. albus</i>	21	11.9	10	20.0	31	13.7
* <i>M. candidus</i>	20	11.4	3	6.0	23	10.2
* <i>M. epidermidis</i>	13	7.4	3	6.0	16	7.1
<i>M. citreus</i>	10	5.7	3	6.0	13	5.7
* <i>M. virians</i>	10	5.7	6	12.0	16	7.1
<i>M. flavus</i>	8	4.5	2	4.0	10	4.4
* <i>M. conglomeratus</i>	4	2.3	5	10.0	9	4.0
* <i>M. luteus</i>	3	1.7	3	6.0	6	2.6
<i>M. casei</i>	2	1.1	0	0.0	2	0.9
Not identified	5	2.8	6	12.0	11	4.9
Total	176	100.0	50	100.0	226	100.0
*Total heat-resistant	71	41.5	30	68.0	101	44.7

\* Indicates heat resistant.

drawn from 161 cows of five different herds in two widely distant sections of the country. Although she stated that the majority of these organisms, while non-virulent, resembled the pyogenic staphylococci (*M. aureus*), she also found *M. caseolyticus* and *M. luteus*, the latter being one of the heat-resistant forms listed above.

From these three studies (66, 67, 68) it seems evident that heat-resistant micrococci make up a significant proportion of the udder flora of normal cows. While their total numbers in the milk in the udder are doubtless small, the farm utensils are constantly inoculated with them. It seems probable that the long tubes of milking machines receive most of their outside contamination from the milk itself. Since these micrococci will withstand heat sterilization unless it is more efficient than is usually the case on farms, and since they will also survive sterilization by chlorine sterilizers and salt brines (63), most farm utensils and many milk cans contain them after they have supposedly been sterilized. If conditions (moisture, nutrients, etc.) are such that growth can subsequently occur, then the utensils will quickly become rich sources of these thermoduric organisms. This accounts, therefore, for their having been found in cans (62) and milking machines (63, 64), and also for the commonly-reported fact that thermoduric organisms are associated with dirty farm utensils, especially milking machines (11, 41, 42, 43, 44, 45, 46, 51, 53, 55, 57, 58).

Those who may be interested in more detailed information concerning the characteristics of the various species of the micrococci are referred to Hucker's "Studies on the Coccaceae", particularly to numbers I, II, III, IV, VIII and IX (69, 70, 71, 72, 35, 65). The staphylococci or parasitic group are considered by Hucker to be species of the genus *microcoecus* (69). In

his latest classification he lists nineteen species (65). Only one, *M. caseolyticus*, causes proteolysis in milk (72).

#### D. ENVIRONMENTAL FACTORS TENDING TO PROMOTE HEAT-RESISTANCE IN BACTERIA

The ability of a given species of bacteria to resist high temperatures is largely a specific characteristic. However, there are certain environmental factors which tend to promote ability to resist heat (and other unfavorable circumstances also), and these will be briefly discussed.

Robertson in 1927 reviewed rather completely the literature on "the thermal resistance of microorganisms" (73). His conclusions are that the thermal resistance of micro-organisms seems to depend to a great degree on the moisture content of the cell. Thus, the moisture content of a spore is lower than that of a vegetative cell, and the cell wall of a spore is less permeable to moisture. Moreover, cells subjected to desiccation survive longer if encapsulated than if not encapsulated. Sarcinae survive desiccation better than certain other species, and they also survive higher temperatures than the micrococci and the common non-spore-forming rods, with the exception of *Microbacterium lacticum* and *Lactobacillus thermophilus*. It is probable that, as suggested by the literature, certain bacteria, when gradually subjected to increasing temperatures, have a faculty of adaptability. This doubtless depends on the elimination of water from the cell contents. Also, suspending cells in distilled water, with lower osmotic pressure than the cell contents and a consequent tendency for water to migrate into the cell, lowers thermal resistance, whereas the reverse is true if cells are suspended or grown in solutions of increasing sucrose concentration. The effect of desiccation by the concentrated sucrose solution is doubtless enhanced by the presence of capsules formed when some species grow in fairly concentrated sucrose solutions.

The effect of either acclimatization or concentration, or both, may be operative in increasing the thermal resistance of bacteria in dairy utensils. Moreover, storage of milking machine tubes in brines may very well serve to strengthen the heat resistance of the organisms by lowering their moisture content.

In another paper in 1927, Robertson (74) reports that cultures of *Microbacterium lacticum*, *Sarcina lutea* and *Streptococcus thermophilus* are more susceptible to heat in the accelerative growth stage than in the resting stage. This doubtless accounts for the observation of Macy (41) that the bactericidal efficiency of pasteurization is greater in summer than in winter. In summer, due to higher temperatures, the organisms may reach the accelerative growth stage before pasteurization, whereas in winter they are doubtless maintained in the resting stage in many cases.

To summarize briefly, it may be stated that certain thermoduric micrococci are normal inhabitants of the bovine udder, so that the milk itself con-

tinually seeds the farm utensils with these organisms. If the utensils are not properly washed and sterilized, these organisms will grow in them, and certain inefficient sterilizing procedures may tend to enhance their thermal resistance. These udder micrococci form a significant proportion (one-third to one-half or even more) of the organisms surviving low-temperature long-hold pasteurization. Moreover, there is reason to believe that they are just as important in milk pasteurized by the high-temperature short-hold method, since at least one paper reports that heat-resistant yellow micrococci are especially abundant on plates made from milk pasteurized by the high-temperature method, and since there seems to be practically universal agreement that cleaning up dirty farm utensils, especially milking machines (which contain large numbers of micrococci), results in a marked decrease in the number of bacteria surviving high-temperature pasteurization. The species of micrococci known to occur in milk and to be heat-resistant are: *M. albus*, *M. candidus*, *M. conglomeratus*, *M. epidermidis*, *M. luteus* and *M. varians*.

Many other species of heat-resistant bacteria have been shown to occur in milk, but their origin is in general not so clearly known as is the origin of the micrococci. Among these organisms may be mentioned the following species, although many of the Bacilli are rather rare: *Bacillus acrothermophilus*, *Bacillus abisolactis*, *Bacillus butyricus*, *Bacillus calidolactis*, *Bacillus calidus*, *Bacillus cereus*, *Bacillus circulans*, *Bacillus glaligu*, *Bacillus kaustophilus*, *Bacillus lacterosporus*, *Bacillus mesentericus*, *Bacillus michaelsii*, *Bacillus mycoides*, *Bacillus nondiastatticus*, *Bacillus panis*, *Bacillus ramosus*, *Bacillus subtilis*, *Bacillus terminalis* var. *thermophilus*, *Bacillus thermoalimentophilus*, *Bacillus thermoliquefaciens*, *Bacillus vulgaris*, *Lactobacillus thermophilus*, *Microbacterium lacticum*, *Sarcina lutea*, *Sarcina rosea*, *Streptococcus bovis*, *S. faecium*, *S. fecalis*, *S. glycoerinaceus*, *S. inulinaceus*, *S. liquifaciens*, *S. thermophilus* and *S. zymogenes*.

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## American Dairy Science Association Announcements

### RESULTS OF ELECTION

The results of the election of officers on October 1, were as follows:

*Vice-President*: HENRY F. JUDKINS, Sealtest Inc., New York, New York.

Directors to serve for three years each: HOWARD B. ELLENBERGER, University of Vermont, Burlington, Vermont; ARTHUR C. DAHLBERG, Agriculture Experiment Station, Geneva, New York.

Annual Meeting—1941—at University of Vermont, Burlington, Vermont, June 23-27.

Many of our members are now making plans along with their families to attend the Annual Meeting next June. Those members who will present papers should write to the Chairman of the Program Committee, Dr. E. S. Guthrie at Cornell University and inform him that you desire to present a paper. Those of you who have been in Vermont will be sure to want to go again. Those who have never been there cannot afford to miss this opportunity.

The Association now has available all the Journals that have been published. You will find the price list for all back Journals in one of the advertising pages.



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## RELATION OF SURFACE TENSION OF RANCID MILK TO ITS INHIBITORY EFFECT ON THE GROWTH AND ACID FERMENTATION OF *STREPTOCOCCUS LACTIS*

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Koestler (1) first observed the inhibitory effect of rancid milk on the growth of *Streptococcus lactis* with the resultant delayed acid fermentation. He noticed that raw rancid milk will not become acid-coagulated even if kept at room temperature for several days. Using milk susceptible to rancidity, he made plate counts on the raw milk and on the milk pasteurized shortly after milking. Some samples were inoculated with *S. lactis* and some were not. The results indicated that the growth-arresting effect of rancid milk is very pronounced. According to Koestler, Roadhouse, and Lörtscher (2), rancid milk significantly inhibits (or slows) the growth of bacteria in milk in general and of *Streptococcus lactis* in particular. As Tarassuk (3) has shown recently, rancidity is one cause for the failure of lactic starters in acid coagulation of milk. The admixture of as little as five per cent of rancid milk in normal milk with the addition of the usual amount of starter gave an acid clot that was very weak. At the higher concentration of rancid milk, the acid coagulation was delayed for as long as eleven hours. Tarassuk found that delayed acid coagulation can be somewhat overcome by increasing the amount of starter added.

The frequency of rancidity in milk can be appreciated from recent contributions by Herrington and Krukovsky (4), Krukovsky and Herrington (5). In many dairy-manufacturing processes, on the other hand, a normal acid fermentation is of utmost importance. Explanation of the phenomenon involved in the inhibition of acid fermentation by rancid milk is, therefore, essential for a rational approach to this problem.

This report will furnish evidence for an explanation of why a rancid milk inhibits lactic-acid fermentation.

### METHODS

The rancid milk used in these studies was one in which a lipase was naturally active—that is, a milk that would become rancid soon after milking without activation measures such as shaking or homogenization. The cow

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producing this milk was free from mastitis, Bang's disease, tuberculosis, and udder deformations.

The surface tension was measured at 20°–21° C. with the Cenco-du-Noüy tensiometer.

Hydrogen-ion concentrations were determined electrometrically with the Bailey hydrogen or quinhydrone electrode.

The bacteriological methods were essentially those described in *Standard Methods of Milk Analysis, sixth edition*.

#### EXPERIMENTAL RESULTS

##### I. Surface Tension of Rancid Milk

Doan and Minster (6) studying the changes in the physical and chemical properties brought about by homogenization of milk, showed that surface tension of homogenized milk is lowered by lipolysis. They attributed this surface tension lowering effect to the formation of appreciable amounts of surface tension active fatty acids. The hydrolysis of fat by lipase was accelerated by homogenization of raw milk. Tarassuk (3) working with milk in which lipase was naturally active has observed the same surface tension lowering effect. As the hydrolytic rancidity develops, the surface tension of milk decreases from a value of 49–51 dynes per cm. to a value of 39 dynes per cm., or even lower. This progressive lowering of surface tension has been confirmed in the present study. (See curve I, fig. 1.) If a milk

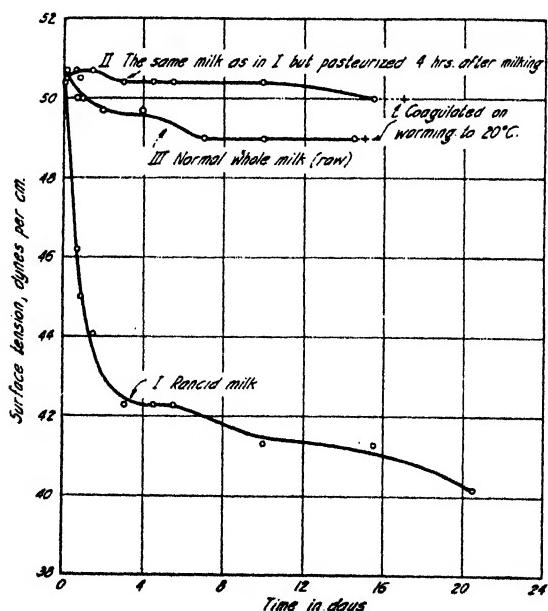


FIG. 1. Change in surface tension of milk on development of rancidity at 7° C.

known to become rancid is pasteurized within a few hours after milking, the surface tension upon aging remains practically constant (curve II, fig. 1).

As compared with a rancid raw milk, the surface tension of a normal milk changes very little throughout the aging period. It decreases as a rule, about one to two dynes per cm. during the first two days and remains fairly constant thereafter. This relatively slight decrease in surface tension may be attributed to (1) a change in the physical state of fat on cooling of milk, as shown by Bauer (7); (2) a slight lipolytic activity which according to Herrington and Krukovsky (4) manifests itself in practically all raw milk.

## II. Relation of Surface Tension of Rancid Milk to Its Acid Coagulation

According to a well-recognized fact, surface tension of media is an important factor in the growth of organisms. Larson, Cantwell, and Hartzell (8) found that a depression of the surface tension would prevent surface growth of pellicle formers. Also, according to these authors, certain anaerobes could grow aerobically when the surface tension was lowered. Wolfe (9), in rather extensive studies on this subject, including several species of bacteria, found certain organisms to be more affected than others.

In the *Lactobacillus* group, the minimum surface tension at which the various species will grow is being used as a means of differentiation.

The work most pertinent to the subject under consideration is that done by Ayers, Rupp, and Johnson (10) on surface tension as affecting various strains of the streptococci. These authors studied several different depressants and several different surface-tension values. Seemingly, a surface tension below 40 dynes per cm. would be necessary to stop the fermentation reactions of *Streptococcus lactis* when inoculated into a carbohydrate medium. Unfortunately, surface tension has not been studied in relation to growth of *Streptococcus lactis* with a milk as a medium of growth.

### (a) Surface tension and acid fermentation of rancid milk aged at various temperatures

In these experiments, milk containing a naturally active lipase was divided into three portions. The respective samples were aged at 7° C., 22° C., and 37° C. The development of rancidity and acid fermentation of the samples was observed by testing surface tension, titratable acidity, and hydrogen ion concentration at intervals of time.

In experiment 1, the samples were placed at their respective temperatures of holding three hours after milking. At this time the milk tested as follows: surface tension 49.7 dynes per cm., titratable acidity 0.12 per cent, and pH 6.83. In experiment 2, the milk was seven hours old, with surface tension of 45.5 dynes per cm., titratable acidity of 0.14 per cent, and pH of 6.69. Tested organoleptically, this milk was already definitely rancid.

Evidently, according to the data in table 1, the rate and extent of lipolysis was greatest when the milk was kept at low temperature. In all cases, the acid coagulation of milk was delayed. The coagulum formed under this

TABLE 1

*Development of rancidity and acid fermentation of rancid milk aged at various temperatures*

Experiment	Aging time	Aged at 7° C.			Aged at 22° C.			Aged at 37° C.		
		S. T.	T. A.	pH	S. T.	T. A.	pH	S. T.	T. A.	pH
number		dynes/cm.	per cent		dynes/cm.	per cent		dynes/cm.	per cent	
1	34 hrs.	41.6	0.13	6.73	43.4	0.15	6.69	46.2	0.55	5.38
	59 hrs.	41.3	0.15	6.68	44.8	0.23	6.10	46.5	0.60	5.24
	83 hrs.	liquid			weak, partial coagulation			weak, partial coagulation		
	6½ days	39.5	0.16	6.63						
	46 days	weak, partial coagulation								
2	28 hrs.	41.3	0.15	6.63	42.7	0.16	6.53	44.8	0.20	6.27
	66 hrs.									
	4 days	39.4	0.17	6.59	44.5	0.44	5.44			
	5 days									
	43 days	41.6	0.32	5.81						

condition is weak, often resembling a precipitate rather than a clot. In experiment 2, the milk aged at 22° C. had stood for over 4 days before acid clot appeared. The same milk aged at 7° C. failed to coagulate on standing as long as 43 days. At the end of this period, in fact, the pH of the milk was 5.81, the titratable acidity 0.32 per cent.

The data in table 1 definitely indicate that the acid fermentation of rancid milk is markedly inhibited; likewise, that the lower the surface tension, the slower the acid fermentation. At favorable growth temperatures, however, not only does the increased acidity check the further development of rancidity (as might be expected), but a rancidity previously produced is decreased, as shown by the rise in surface tension.

(b) *Surface tension and acid fermentation of rancid milk when inoculated with Streptococcus lactis*

In these experiments a portion of the milk containing active lipase was pasteurized at 60° C. for 30 minutes within 3-6 hours after milking. Samples of the pasteurized and raw milk inoculated with a pure culture of *Streptococcus lactis* were incubated at 30° C. The data in table 2 show definitely the inhibition of growth of *Streptococcus lactis* in part of a sample of milk in which lipase was allowed to remain active. These studies confirm also the previous suggestion that when the growth of *Streptococcus lactis* in rancid milk reaches a certain stage, the surface tension of milk increases.

(c) *The growth and acid fermentation of Streptococcus lactis in sterilized rancid milk and their relation to the surface tension of the milk*

That the phenomenon of delayed acid fermentation of rancid milk is

TABLE 2

*Development of rancidity and acid fermentation in lipolytically active raw milk and in the same milk pasteurized. Both milks inoculated with *Streptococcus lactis**

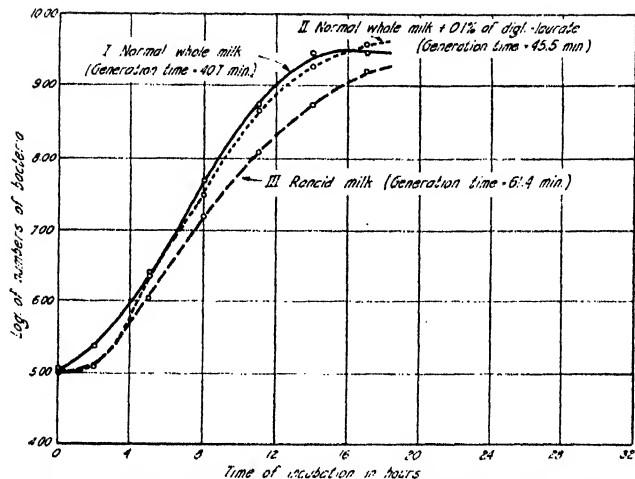
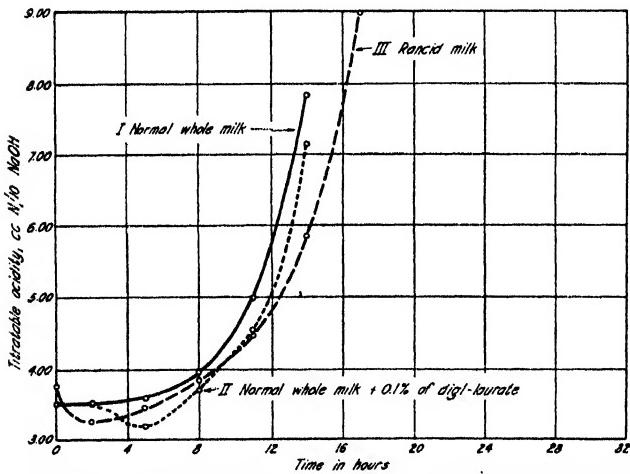
Sample	Time of incubation	Plate count	Surface tension	Titratable acidity	pH	Remarks
(a) Raw milk—4 hrs. after milking	hours immediately after inoculation	133,000	dynes/cm. 49.7	per cent 0.14	6.67	
(b) Milk (a) pasteurized	"	144,000	50.0	0.14	6.68	
(a) Raw milk	20	500,000,000	45.5	0.20	6.13	
(b) Pasteurized	20	1,180,000,000	50.0	0.25	5.90	
(a) Raw milk	28	.....	.....	.....	.....	Liquid Coagulated
(b) Pasteurized	28	.....	.....	.....	.....	
(a) Raw milk	33	.....	46.2	0.54	5.41	Liquid Coagulated
(a) Raw milk	35	.....	.....	.....	.....	

caused by a low surface tension of such milk is again demonstrated in the following experiments. Whole milk containing a naturally active lipase was allowed to stand in the cold until it became very rancid. During this time the surface tension decreased from 50.7 dynes per cm. to 41.3 dynes per cm. Using the rancid milk and fresh normal milk, the following samples were made: I. normal whole milk, II. normal whole milk + 0.1 per cent of diglycol laurate, and III. rancid whole milk. All samples were sterilized in an autoclave at 115° C. for 20 minutes. After cooling they were inoculated with *Streptococcus lactis* and incubated at 30° C. At frequent intervals during the incubation period, samples were examined to determine the numbers of bacteria, the titratable acidity, the surface tension, and the time of coagulation. The results appear in figures 2, 3, and 4.

The reason for inoculating the sterile samples of milk with *Streptococcus lactis* was as follows. If the surface tension is the factor in the phenomenon of delayed acid fermentation of rancid milk, then a sterile rancid milk inoculated with *Streptococcus lactis* should also exhibit the same phenomenon, provided that sterilization does not materially change the surface tension of rancid milk. Actually, we found that sterilization of rancid milk raises its surface tension only one to two dynes per cm. Furthermore, a normal milk whose surface tension is lowered by means other than lipolysis should behave like a rancid milk in respect to acid fermentation. It is for this reason that a diglycol laurate, a powerful surface-tension depressant oil,\* was added to sample II.

As is evident from figure 2, the growth of *Streptococcus lactis* in milk

\* It is advertised by a manufacturer as a non-toxic, edible oil.

FIG. 2. Growth of *Streptococcus lactis* in sterilized rancid milk.FIG. 3. Change in titratable acidity with growth of *Streptococcus lactis* in sterilized rancid milk.

with a low surface tension is markedly inhibited. This inhibition is true whether caused by the addition of a surface-tension depressant or by a low surface tension resulting from a hydrolysis of milk fat. As the growth curves of *Streptococcus lactis* show, when the surface tension is low there is a longer lag period, together with a marked increase in the generation time in the period of logarithmic growth. Along with the inhibition of growth one finds a marked delay in acid fermentation. This latter fact is shown by the curves depicting the change in titratable acidity presented in figure 3.

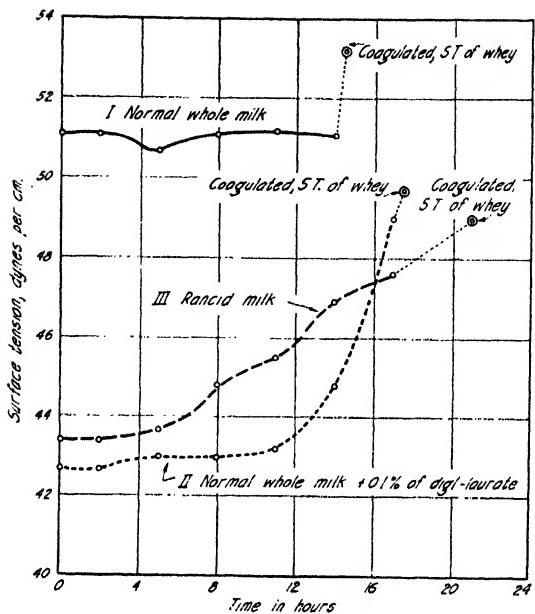


FIG. 4. Change in surface tension with growth of *Streptococcus lactis* in sterilized rancid milk.

The unexpected and most interesting detail in this graph is the decrease in titratable acidity in samples II and III during the first four to five hours of growth. The reason for this decrease becomes apparent after we study in figure 4 the change in surface tension brought about by growth of *Streptococcus lactis*. During the acid-fermentation period resulting from the growth of *Streptococcus lactis*, the surface tension of a normal milk remains essentially unchanged. On the other hand, in rancid milk and normal milk with depressed surface tension, the surface tension begins to increase after the first two hours of incubation. This increase becomes very appreciable during the logarithmic stage of growth; and when acid fermentation reaches the coagulation stage, the surface tension of these samples of milk approaches the surface-tension value of a normal milk. The change in surface tension, therefore, must come through a change in the depressant. In rancid milk, then, the fat acids responsible for the lowering of surface tension are utilized by *Streptococcus lactis* in the process of growth. Thus, a decrease in titratable acidity in the first stage of growth becomes self-apparent.

In a separate experiment in which lauric acid was used as a surface-tension depressant, similar results in respect to changes in surface tension and titratable acidity were obtained.

The ability of *Streptococcus lactis* to utilize certain fat acids in the process of growth and thus to raise the surface tension of rancid milk is

confirmed also by the following fact. The surface tension of whey obtained from rancid whole milk by a direct precipitation with acid is normally higher by two or three dynes per cm. than that of milk itself. In contrast, the surface tension of the whey obtained from a rancid milk as a result of *Streptococcus lactis* fermentation, may be as much as ten dynes per cm. higher than that of milk itself prior to fermentation.

#### CONCLUSIONS

1. Inhibitory effect of rancid milk on the growth and acid fermentation of *Streptococcus lactis* has been confirmed.
2. The inhibitory effect has been shown to result from a low surface tension of rancid milk.
3. Appreciable growth of *Streptococcus lactis* in rancid milk increases the surface tension of the milk. Under optimum conditions of growth in respect to temperature of incubation and initial number of organisms, this increase in the surface tension of rancid milk yields a final surface-tension value approaching that of normal milk. The change in surface tension apparently results from the utilization of surface-tension-lowering fat acids by *Streptococcus lactis* in the process of growth.

#### ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. G. A. Richardson for helpful suggestions and criticism of certain parts of the manuscript.

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## AGE AS A FACTOR INFLUENCING BREEDING EFFICIENCY IN A DAIRY HERD\*

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One of the objections to the exclusive use of proved sires in a dairy herd pertains to the difficulty of getting heifers safe in calf. The general opinion is that a greater number of services is required per conception in heifers when bred to mature bulls than when bred to younger bulls. Breeding efficiency in dairy cattle is of considerable economic importance. This is especially true where dairymen are making an effort to maintain a uniform production level, or attempting to have their cows and heifers calve during a certain period in order to establish a base production level that will meet the marketing situation prevalent in many milk sheds.

The data that have accumulated in the herd books of the West Virginia Agricultural Experiment Station have been studied with the hope that some light might be thrown on the influence of age on the breeding efficiency of dairy cattle. A preliminary report (1) indicated a smaller number of services per conception in heifers when bred to young bulls than when bred to older bulls. These results checked with the conclusions of Morgan and Davis (2) based on a similar study in the Nebraska Station herds. In order that more definite conclusions might be drawn in regard to this question, a further analysis was made of the data at hand with special reference to the influence of the age of the bull and the age of the female upon the number of services required per conception in dairy cattle.

### PROCEDURE

The data for this study were taken from the herd books of the Dairy Department of the West Virginia Agricultural Experiment Station for the period from January 1, 1920 to August 1, 1938. Only those animals which had given birth to calves, or which were known to be breeders, were considered. All animals which were known to be non-breeders were discarded from the data and were not used in tabulating the results.

Since all of the Ayrshires and a large percentage of the cows of the other breeds were negative to the Bang's test no attempt was made to study the two groups separately and both were treated as one group.

The bulls ranged in age from one to fifteen years. The ages of the bulls were calculated to the nearest year. The ages of the females were considered on the basis of conception since the conception seemed to be more important than actual age in this study.

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The females were grouped by conceptions ranging from one to six. However, due to the small number of cows in the sixth group it was considered advisable to combine the fifth and sixth conceptions and treat them as one. The number of animals beyond the sixth group was so small it was not considered. The study included a total of forty-three males and seven hundred six females grouped as shown in table 1.

TABLE 1  
*Arrangement of total number of females according to breed and conception*

Conception	Breed of cattle			
	Ayrshire	Holstein	Jersey and Guernsey	Total
First .....	420	131	80	631
Second .....	322	106	69	497
Third .....	205	71	48	324
Fourth .....	123	45	32	200
Fifth and Sixth .....	106	38	26	170

#### RESULTS

Table 2 is a summary of the results of using bulls of all ages on females of all ages. Where no consideration was given either to the age of the bull or to the age of the female to which he was mated, the heifers that were bred for first calving required a larger number of services per conception than did the females of any other group. After the first conception, however, the number of services per conception did not vary to a marked degree.

TABLE 2  
*Average number services per conception using bulls of various ages on females of all ages*

	Conception					
	1st	2nd	3rd	4th	5th and 6th	Total
Number of Females .....	631	497	324	200	170	
Number of Services .....	1601	894	572	354	289	3710
Number of Conceptions ..	631	497	324	200	170	1822
Services per Conception ..	2.79	1.86	1.79	1.82	1.80	2.02

When the data are divided to show the influence of the age of both the bulls and the females a more detailed picture of the results can be seen. This material is presented in table 3. It is apparent that the age of the bull has a significant effect upon the number of services per conception in the case of heifers. Bulls under four years of age when bred to heifers showed a greater efficiency than bulls of any other age. In the case of the females bred for the second conception there was also a small but significant differ-

ence in favor of the bulls under four years of age. Beyond the second conception there was no significant effect of the age of the bull.

In the case of cows of all ages bred to bulls under four years of age there is not a marked difference in the number of services per conception, except in the case of uncalved heifers. In all but three of the eleven age intervals for bulls above four years, however, a larger number of services per conception was required in the case of first conceptions than for any other conceptions.

Figures 1 to 5 inclusive show the regression lines and the regression

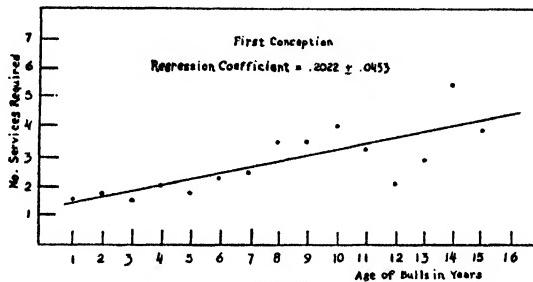


Figure 1

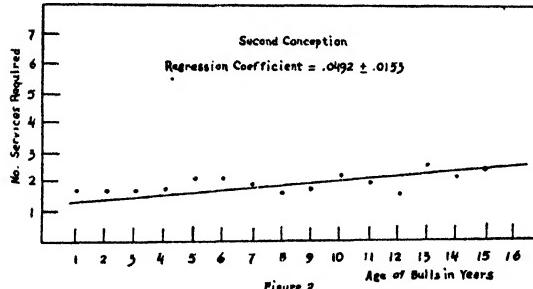


Figure 2

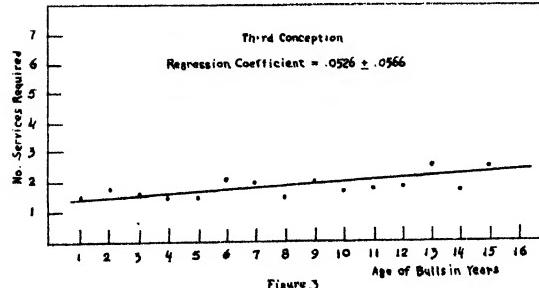


Figure 3

coefficients when the number of services for the various conceptions are plotted against the age of the bulls. The regression coefficients were plotted by the method of least squares.

The regression coefficients for figures 1 and 2 are highly significant. The regression coefficients for the remaining figures are not significant but the lines show a tendency for the breeding efficiency to decrease as the age of the bull increases. Figure 6 shows a comparison of the services required

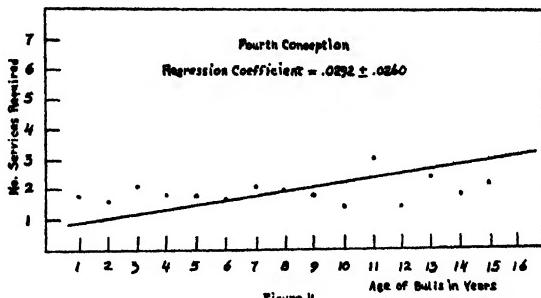


Figure 4

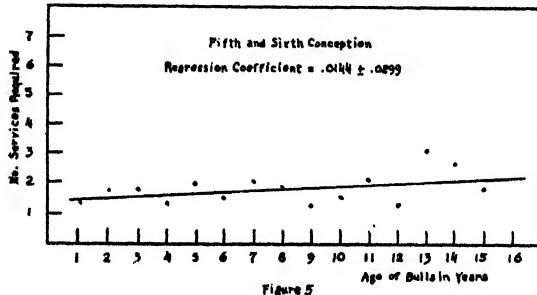


Figure 5

TABLE 3

*Effect of age of bulls upon services per conception with females of different ages (conceptions)*

Age of bulls	Conception					Mean
	1st	2nd	3rd	4th	5th & 6th	
1- 2 years ..	1.49	1.58	1.38	1.72	1.33	1.50
2- 3 " ..	1.62	1.45	1.76	1.57	1.76	1.63
3- 4 "	1.53	1.45	1.51	2.00	1.77	1.65
4- 5 "	1.97	1.58	1.46	1.58	1.22	1.56
5- 6 "	1.79	2.09	1.42	1.73	1.92	1.79
6- 7 "	2.23	2.04	2.00	1.53	1.57	1.87
7- 8 "	2.42	1.82	1.93	2.08	2.00	2.05
8- 9 "	3.56	1.61	1.41	1.84	1.81	2.04
9-10 "	3.50	1.72	1.96	1.72	1.20	2.02
10-11 "	4.06	2.06	1.60	1.25	1.58	2.10
11-12 "	3.27	1.88	1.77	3.00	2.00	2.38
12-13 "	2.00	1.52	1.83	1.25	1.20	1.56
13-14 "	2.97	2.52	2.52	2.25	3.14	2.68
14-15 "	5.50*	2.17	1.68	1.76	2.66	2.76
15-16 "	3.90	2.30	2.58	2.00	1.80	2.57
Mean .....	2.79	1.85	1.79	1.82	1.79	

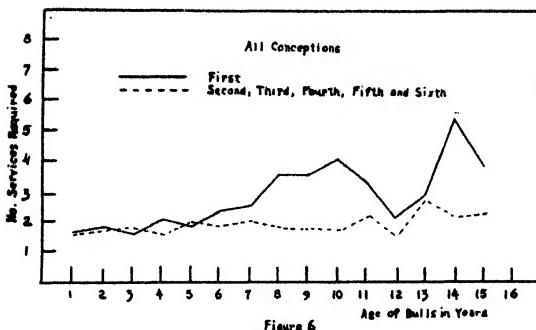


Figure 6

for first conceptions with the average number of services required for conceptions at all ages.

TABLE 4  
*Analysis of variance of services per conception for animals in different age groups*

Source of variation	Degrees of freedom	Sums of squares	Variance
Total .....	74	40.460	.546
Between conceptions .....	4	11.419	2.855**
Between ages of bulls .....	14	12.049	.861**
Discrepance .....	56	16.992	.303

Least significant mean difference between conceptions ..... .68

Least significant mean difference between ages ..... .40

\*\* Highly significant.

#### SUMMARY

Seven hundred and six females and forty-three males are included in this study. A summary of their breeding records shows that when no consideration was given to the age of the bull the number of services required for the first conception was significantly higher than for the following conceptions. Uncalved heifers required a smaller number of services per conception when bred to bulls under four years of age than when bred to older bulls.

There was a gradual decrease in the breeding efficiency of bulls as they increased in age, but when used on females of all ages this decreased efficiency, when compared to the yearling age, did not become significant until the bulls reached an average age of six years. Bulls 13 years old and over were significantly less sure as breeders than bulls six years of age.

It appears from this study that the use of old bulls is an important factor in the breeding efficiency of dairy heifers, and that dairymen may be

justified in mating heifers with young bulls in order that prompt conceptions may result.

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## A SEMIMICRO-KJELDAHL METHOD FOR THE DETERMINATION OF TOTAL NITROGEN IN MILK

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Recent research work in this laboratory pertaining to the nitrogen distribution in dairy products made it desirable to develop a practical method for determining small amounts of nitrogen.

A survey of the literature disclosed a number of methods designed for this purpose, but after careful consideration of the time factor, routine applicability and accuracy, the semimicro-Kjeldahl procedure used by Rowland (1) was chosen as the most practical method for the analysis of milk.

Rowland does not describe the distillation apparatus he used, but stated that direct steam distillation was utilized to liberate ammonia from the digested samples. He used selenium oxychloride in the digestion mixture because it markedly reduced the time for digestion, increased the amounts of nitrogen determined and improved the agreement of duplicates.

The results this author reported for the total nitrogen in milk are not compared with the Official Method (2), but he stated that duplicate determinations agreed within  $\pm 0.2$  per cent nitrogen.

The purpose of this experiment was threefold: (a) to design a semimicro-Kjeldahl apparatus, (b) to compare the efficiency of several digestion catalysts and (c) to compare both standard acid and boric acid as the ammonia receiving agents.

### DESCRIPTION OF APPARATUS

The apparatus shown in figure 1 proved to be very efficient. The ammonia is liberated from the digested sample by direct steam distillation through a small distillation bulb and a 250 mm. condenser which has a pyrex center tube. Steam is furnished by heating a 3 liter flask containing distilled water slightly acidified with sulphuric acid.

### REAGENTS USED

#### (1) 50 per cent Sodium Hydroxide Solution

500 gms. U.S.P. NaOH, 500 ml. of distilled water and 125 gms. of sodium thiosulphate.

#### (2) Standard Sulphuric Acid and Sodium Hydroxide Solution

The acid and alkali solutions used for titrations were 0.02 N.

#### (3) Boric Acid Solution

A stock solution contained 1 pound of boric acid to 10 liters of distilled water. This solution was diluted (2 parts to 3 of water) and 25 ml. used to receive the ammonia.

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**(4) Indicator**

100 mg. of methyl red and 30 mg. of methylene blue dissolved in 60 ml. of 95 per cent ethyl alcohol and made up to 100 ml. with distilled water.

**(5) Catalysts**

Three catalysts were used in the following experiments:

- (a) 0.2 gm. copper sulphate and 2 drops of selenium oxychloride.
- (b) 0.14 gm. HgO and 2 drops of selenium oxychloride.
- (c) 0.14 gm. HgO only.

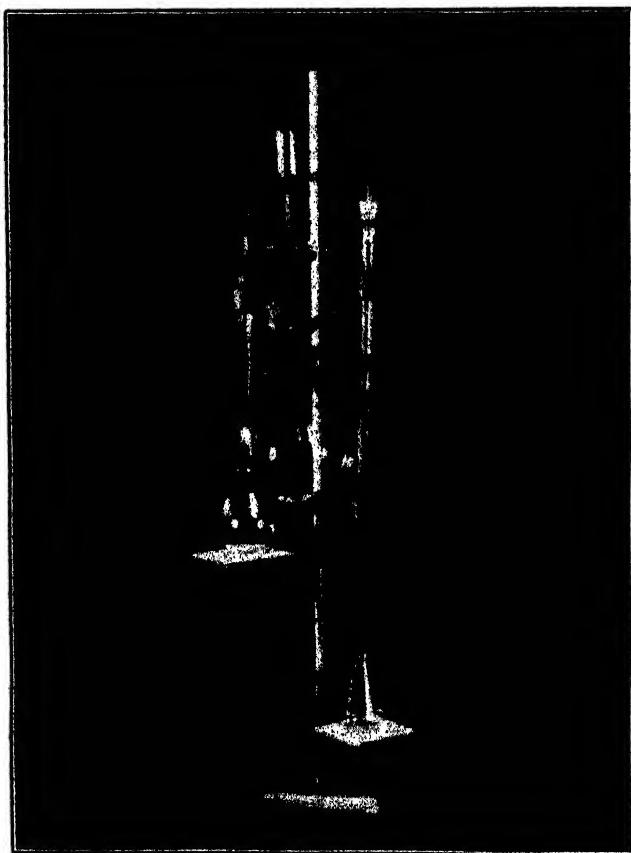


FIG. 1. Semimicro-Kjeldahl distillation apparatus.

**PROCEDURE**

Approximately five grams of milk, at room temperature, were weighed with a Mojonnier weighing cross and 5 gram pipets. The pipets were emptied into 100 ml. volumetric flasks and these filled to the mark with

distilled water. The samples were thoroughly mixed and 20 ml. of each pipetted into 300 ml. Kjeldahl digestion flasks. The different catalysts were added in the proportions mentioned above. Two gm. of sodium sulphate and 5 ml. of nitrogen-free sulphuric acid were then added and the samples containing selenium were digested over a moderate flame for 40 to 45 minutes or about fifteen minutes after becoming clear. The samples containing HgO only as a catalyst were digested for the same length of time but with a slightly hotter flame.

After digestion 50 ml. of distilled water was added to each sample. Fifteen ml. of the 50 per cent NaOH solution was added and the samples steam-distilled for approximately 10 minutes, the distillate passing into a 125-ml. Erlenmeyer flask containing 25 ml. of 0.02 N acid to which 3 or 4 drops of the methyl red—methylene blue indicator had been added. The indicator was added with a 0.1-ml. pipet. In some experiments 25-ml. of boric acid solution, containing the same amount of indicator, was used to receive the ammonia.

Just before starting a series of distillations a clean Kjeldahl flask containing a little distilled water was steam-distilled until the walls of the condenser and the distillation bulb were covered with moisture. This was done to prevent any loss of ammonia in the air discharged from the system at the beginning of the distillation.

All samples were distilled so that 60 to 70 ml. of the distillate collected in approximately 10 to 12 minutes. A 10-ml. buret graduated to 0.05 ml. was used for making these titrations. Duplicate blank determinations of nitrogen in the reagents were made with each set of samples.

The following experiments show the recovery of nitrogen from urea and the determination of protein ( $N \times 6.38$ ) in various samples of milk. All results are compared with those obtained by the Official Kjeldahl-Gunning-Arnold Method (2) and this will be referred to as the Official Method.

The following tables show the recovery of nitrogen from urea using all three catalysts and with standard acid and boric acid as the ammonia receiving agents. For the semimicro-Kjeldahl 240 mg. of urea were dissolved in 500 ml. of distilled water and 25 ml. taken for a sample. For the Official Method 1.2 gm. of urea were dissolved in 500 ml. of distilled water and 25 ml. taken for a sample.

The Official Method gave a recovery of 27.50 mg. of nitrogen from the 25 ml. samples used in the determinations. On the basis of this recovery 5.50 mg. of nitrogen should be recovered from each semimicro sample. The percentage of nitrogen recovered in the following tables is computed on the basis of this value (5.50 mg. of nitrogen per sample).

The following samples were distilled into standard acid and back titrated with standard alkali.

TABLE 1

*Recovery using 25 ml. of standard acid as the ammonia receiving agent and titrating with standard alkali*

Se and CuSO <sub>4</sub>	Recovery	Se and HgO	Recovery	HgO	Recovery
	<i>per cent</i>		<i>per cent</i>		<i>per cent</i>
5.46	99.27	5.44	98.90	5.38	97.82
5.40	98.18	5.34	97.09	5.47	99.45
5.43	98.73	5.11	92.90	5.45	99.09
5.36	97.45	5.52	100.36	5.36	97.45
5.50	100.00	5.34	97.09	5.40	98.18
5.45	99.09	5.39	98.00	5.57	101.27
5.46	99.27	5.42	98.55	5.40	98.18
5.48	99.64	5.50	100.00	5.45	99.09
5.40	98.18	5.43	98.73	5.47	99.45
5.49	99.82	5.47	99.45	5.32	96.73
Av. 5.45	99.09	5.40	98.18	5.43	98.73

TABLE 2

*Recovery using 25 ml. of boric acid solution as the ammonia receiving agent and titrating direct with standard acid*

Se and CuSO <sub>4</sub>	Recovery	HgO	Recovery
	<i>per cent</i>		<i>per cent</i>
5.56	101.09	5.53	100.55
5.57	101.27	5.49	99.82
5.58	101.45	5.58	101.45
5.51	100.08	5.47	99.45
5.54	100.73	5.58	101.45
5.51	100.08	5.58	101.45
5.54	100.73	5.35	97.27
5.54	100.73	5.37	97.60
5.60	101.82	5.46	99.27
5.48	99.64	5.36	97.45
Av. 5.54	100.73	5.48	99.64

TABLE 3

*Analysis of homogenized milk with boric acid as the receiving agent  
(Official Method—3.41 per cent protein)*

Se and CuSO <sub>4</sub>	*	Se and CuSO <sub>4</sub>	*	HgO	*	HgO	*	HgO	*
3.38	-0.03	3.41	0.00	3.37	-0.04	3.44	+0.03	3.43	+0.02
3.34	-0.07	3.38	-0.03	3.31	-0.10	3.40	-0.01	3.44	+0.03
3.41	0.00	3.41	0.00	3.30	-0.11	3.44	+0.03	3.42	+0.01
3.40	-0.01	3.32	-0.09	3.42	+0.01	3.45	+0.04	3.44	+0.03
3.39	-0.02	3.40	-0.01	3.43	+0.02	3.42	+0.01	3.33	-0.08
3.27	-0.14	3.38	-0.03	3.35	-0.06	3.46	+0.05	3.43	+0.02
3.40	-0.01	3.40	-0.01	3.43	+0.02	3.42	+0.01	3.44	+0.03
3.30	-0.11	3.40	-0.01	3.43	+0.02	3.40	-0.01	3.45	+0.04
Av. 3.36	-0.05	3.39	-0.02	3.38	-0.03			3.43	+0.03

\* The difference between the semimicro values for total protein and the Official Method.

TABLE 4  
*Analysis of homogenized milk (Official Method—3.51 per cent protein)*

Recovery using standard acid				Recovery using boric acid			
Se and CuSO <sub>4</sub>	*	HgO	*	Se and CuSO <sub>4</sub>	*	HgO	*
3.68	+ 0.17	3.38	- 0.13	3.45	- 0.06	3.53	+ 0.02
3.39	- 0.12	3.42	- 0.09	3.33	- 0.18	3.49	- 0.02
3.37	- 0.14	3.46	- 0.05	3.42	- 0.09	3.50	- 0.01
3.48	- 0.03	3.45	- 0.06	3.45	- 0.06	3.52	+ 0.01
3.46	- 0.05	3.42	- 0.09	3.45	- 0.06	3.50	- 0.01
3.39	- 0.12	3.39	- 0.12	3.54	+ 0.03	3.44	- 0.07
3.40	- 0.11	3.44	- 0.07	3.49	- 0.02	3.51	0.00
3.45	- 0.06	3.34	- 0.17	3.51	0.00	3.52	+ 0.01
Av. 3.45	- 0.09	3.41	- 0.10	Av. 3.46	- 0.06	3.50	- 0.009

\* See table 3.

Samples of abnormal milk were sent to the laboratory for complete analysis so it was possible to calculate the percentage of protein by difference in addition to making semimicro and macro analyses for protein. All three catalysts were used in these experiments and standard acid used to receive the ammonia. The samples in these experiments are all from the

TABLE 5  
*A comparison of different methods of determining protein*  
 Experiment 1

Sample No.	Protein Semimicro Se & CuSO <sub>4</sub>	Protein Official	Protein by difference		
	per cent	per cent	per cent		
587	3.28	2.98	3.34		
573	3.65	3.64	3.64		
746	3.18	3.23	3.20		

Experiment 2

Sample No.	Protein Semimicro Se & HgO	Protein Semimicro HgO	Protein Official	Protein by difference	
	per cent	per cent	per cent	per cent	
587	3.19	3.20	3.24	3.24	
573	3.65	3.65	3.61	3.61	
746	3.23	3.32	3.34	3.33	

Experiment 3

Sample No.	Protein Semimicro Se & CuSO <sub>4</sub>	Protein Semimicro Se & HgO	Protein Semimicro HgO	Protein Official	Protein by difference
	per cent	per cent	per cent	per cent	per cent
587	3.43	3.39	3.42	3.42	3.43
573	3.91	3.90	3.93	3.92	3.89
746	3.27	3.31	3.33	3.18	3.22

same cows but each experiment is a different set of samples. The results tabulated in table 5 are the averages of duplicate determinations.

The data presented in table 6 show the approximate digestion time required for each catalyst. The gas burners were adjusted to a moderate flame and not changed until the digestion of all the samples was completed. Samples were digested 15 minutes after clear and standard acid used as the receiving agent.

TABLE 6

*The effect of the catalyst on the digestion time required (Official Method—3.43 per cent protein (Homogenized milk))*

Catalyst	Per cent protein found after digesting				
	20 min.	30 min.	40 min.	50 min.	60 min.
Se & CuSO <sub>4</sub> .....	.....	3.39	3.44	3.44	3.44
Se & HgO .....	3.41	3.45	3.46	3.45	3.41
HgO .....	.....	3.39	3.44	3.46	3.43

Some investigators (3, 4) report the loss of ammonia when samples containing selenium are subjected to a prolonged digestion. In the following experiment (table 7) four burners were adjusted so that each burner gave a hotter flame than the preceding one. The first sample, shortest digestion time, was heated to extremes. All samples were digested until 15 minutes after clear. Boric acid was used as the receiving agent.

TABLE 7

*The effect of the catalyst on the digestion time required when the intensity of the heat was varied (Official Method—3.41 per cent protein (Homogenized milk))*

Se & CuSO <sub>4</sub>		Se & HgO		HgO	
Digestion time	Protein	Digestion time	Protein	Digestion time	Protein
min.	per cent	min.	per cent	min.	per cent
20	3.37	25	3.34	30	3.40
30	3.39	30	3.40	32	3.43
40	3.36	38	3.43	40	3.37
55	3.41	38	3.43	47	3.44

In the last two results in the columns above the burners were adjusted about the same as the adjustment used in the regular digestion procedure. There seems to be little effect by heating the samples excessively.

The catalyst containing selenium oxychloride and HgO seems to be slightly more efficient than the other catalysts in this and especially in the previous experiment.

#### DISCUSSION

In the data presented no distinct differences can be noted in the nitrogen and protein values obtained by using the various catalysts.

Rowland (1) in his original report stated that the addition of selenium to the digestion mixture increased the amount of nitrogen determined, but his comparison was made between copper sulphate with and without the addition of selenium.

It is not surprising that the addition of selenium increased the nitrogen values because copper sulphate seems to be inefficient for digesting milk protein. The authors have found that copper sulphate alone is a slower catalyst than HgO or metallic mercury and it has a tendency to cause low results for total nitrogen if the same digestion time is used for all three catalysts.

Lauro (5) used selenium alone as a catalyst because of its digestion efficiency. He also considered it a desirable catalyst because no precipitating agent is required. Most of the data pertaining to the use of selenium as a catalyst has been obtained by the analysis of cereal grains or their products and these results are somewhat conflicting (4, 6, 7).

Poe and Nadler (8) state that a combination of Cu, selenium and Hg effects the greatest saving of time for digestion. Prince (9) claims selenium alone has no advantage over mercury but when they are used together the digestion time is lessened one half. Osborn and Krasnitz (10) have somewhat the same opinion. They found that mercuric oxide and selenium is a more effective catalyst than selenium and copper sulphate. They conclude that when the digestion period is extended the danger of the loss of nitrogen increases in the following order: mercury, selenium and copper sulphate, and mercuric oxide and selenium. They believe that the danger from the loss of nitrogen may be reduced by using larger amounts of acid.

Davis and Wise (6) logically state that the indications favor a lower result with the use of selenium but this may be compensated for by careful research by each collaborator into the time of digestion, intensity of heat applied and the amount of sulphate used in the digestion.

The above discussion would lead to the conclusion that selenium would probably not be as universally used as metallic mercury or mercuric oxide. The literature for the most part, in spite of conflicting evidence, indicates that low results are experienced with the use of selenium or its combinations.

Because of this criticism of selenium the writers prefer to use HgO. This gives results as satisfactory as any of the selenium combinations.

Rowland used 0.02 N acid to receive the ammonia and boiled the distillates to remove carbon dioxide. After cooling, the samples were titrated with 0.02 N NaOH using 0.1 per cent methyl red solution as the indicator.

This procedure is not well adapted to routine analysis and the endpoint in the titration is not entirely satisfactory because it is not well defined.

The use of the boric acid solution as the receiving agent for the ammonia is preferred because it eliminates boiling the samples to remove carbon dioxide. The solution does not have to be measured accurately; it elimi-

nates the use of the dilute NaOH which requires frequent restandardization and is very sensitive to carbon dioxide. The direct titration with standard acid also simplifies calculations. In the previous experiments slightly higher and more consistent results have been obtained with the boric acid solution than with the standard acid and alkali.

Methyl red is not satisfactory for the direct titration of boric acid because the endpoint is indefinite. The methyl red-methylene blue indicator gives a distinct endpoint and has the advantage that the two components of this indicator may be adjusted so that the end point will satisfy the individual analyst. This combination indicator is also satisfactory if standard acid is used as the ammonia receiving agent.

As mentioned under "Procedure" (page 2) a flask containing distilled water was steam distilled before starting the samples to prevent the loss of ammonia. Redemann (11) claims that some of the error in the semimicro determinations is due to the loss of ammonia in the air forced out at the beginning of the distillation of a sample. For this reason the above procedure was adopted. It seemed reasonable to expect no loss of ammonia if the system contained moisture because of the water solubility of this compound and the small amount of nitrogen present in the semimicro sample (0.5 per cent approximately). This theory was checked by using some of the urea solution used in table 1 and 25 ml. of distilled water as the ammonia receiving agent. In the following samples selenium and copper sulphate were used as the catalysts. *N Recovered*, Mg 5.57, 5.55, 5.46, 5.52, 5.55, 5.48, 5.37, 5.47, 5.62, 5.55—Av. 5.51.

These results prove that very little if any of the ammonia is lost if some moisture is present on the walls of the system at the beginning of the distillation.

The semimicro method presented may be modified in many ways by the analyst and still give accurate results; however, its main advantage is the saving of time and the reduced cost of reagents per determination.

#### SUMMARY

1. A semimicro-Kjeldahl method and apparatus is described.
2. Mercuric oxide is recommended as a catalyst with boric acid as the ammonia receiving agent and methyl red-methylene blue as the indicator.
3. The semimicro method was found to check very closely with the Official Method.
4. The semimicro method is useful for determining small amounts of nitrogen, it is time saving, well adapted to routine analysis, and reduces the cost of reagents per determination.

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# A STUDY OF FRESH AND FROZEN PLAIN, SUPERHEATED, AND SWEETENED CONDENSED SKIMMILK FOR ICE CREAM<sup>1</sup>

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## INTRODUCTION

A method of storing milk solids in the form of frozen condensed skimmilk has been tried in the University of Nebraska Creamery and in general has proved successful. However, there have been times when the protein in the condensed skimmilk, which has been held frozen in storage, has become precipitated and gelation has occurred. These changes in the protein have not proved serious, but if the frozen condensed skimmilk is to be sold, it is necessary to reprocess it, which involves added expense and increases the cost of the milk solids.

In the freezing of milk and cream, viscosity as well as the factor of time plays a significant part in limiting the aggregation of colloidal particles. In the case of frozen cream to which sugar has been added, the point where viscosity limits the destabilizing effect of freezing is reached early and aggregation of the colloidal particles is reduced. On the basis of such an explanation it is reasonable to assume that similar results could be obtained by adding sugar to condensed skimmilk which is to be held frozen in storage. It has been shown by Pyenson and Dahle (12) that superheating of condensed skimmilk to 180° F. for 20 minutes brings about an increased stability of the proteins, as measured by the alcohol number, and that the large increase in viscosity due to superheating is associated with the coagulation of calcium caseinate and is not due to hydration. With this in mind, it was desired to include superheated condensed skimmilk in this study to observe the effects of freezing and storing on this type of condensed skimmilk. It seemed logical to believe that if condensed skimmilk was to be stored during the season of surplus and used during shortage, a three-months storage period would be sufficient, and it was upon this basis that the problem was studied.

The purpose of this problem was to study the effects of freezing and storing upon plain condensed skimmilk, superheated condensed skimmilk and sweetened condensed skimmilk, and to compare the advantages of one type over another as a possible means of storing serum solids for ice cream manu-

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facture. Any defects in the frozen condensed skimmilk which might add to the difficulties of incorporation in the ice cream mix, or which might require the condensed to be reprocessed, were particularly observed, as were any changes in the quality of the finished ice cream. Changes in the whipping ability of the ice cream mixes and any other variations resulting from the use of the three types of fresh and stored frozen condensed skimmilk were carefully observed.

#### REVIEW OF LITERATURE

Webb and Hall (17) found that fresh whole milk could be pasteurized, condensed to one-third its weight, canned and frozen without any detrimental effects to the body or flavor of the product. Such a milk, if held at a temperature below -13° (8.6° F.), could be thawed and reconstituted with cold water to yield a product similar to normal fresh milk at any time up to the fourth week of storage. They stated that high heat treatment, long-time heat treatment or high storage temperature shortens the storage period in which the milk is free from casein precipitation.

Doan and Featherman (7) found that in concentrating and freezing milk for storage purposes, a degree of concentration of approximately three to one appears to give the most satisfactory results. Milk of such a concentration could be stored up to 12 weeks and still produce a normal reconstituted product.

Anderson and Pierce (1) showed that holding milk at -25.7° C. (-14° F.) gave no precipitated protein until the third month of storage and was usable until the sixth month. Milk held at -12.5° C. (10° F.) showed some protein precipitation at the end of the second month, and at the end of the fifth month practically all of the protein had precipitated. Referring to the work of Webb and Hall, Doan and Baldwin (6) stated that freezing, itself, has no measurable effect upon protein dispersion and that holding milk in the frozen condition for several weeks or months is required to cause aggregation, denaturation, or instability of the proteins. Mack (8) found that the proteins of ice cream mixes made from frozen cream were less stable than those of the mixes made from fresh sweet cream, as measured by the protein stability test used by Doan (3).

While studying factors affecting the bound-water content of some dairy products, Dahle and Pyenson (2) found that holding skimmilk for long periods of time in the frozen state had no great effect upon alcohol stability of the proteins, but with condensed skimmilk destabilization of the proteins occurred. The condensed skimmilk increased in bound water when stored in a frozen condition for 25 days but after 57 days a marked precipitation had occurred. Doan (5) concluded that when frozen condensed whole milk is stored more than 8 weeks, the proteins become denatured and gelation occurs when the frozen condensed milk is thawed. Munkwitz, Berry, and

Boyer (9) stated that "freezing of milk causes a partial precipitation of milk solids, albumen being precipitated in the greatest amount, followed in order by lactose, total protein, ash, casein, total solids, and fat. With the exception of fat, the amount of precipitation of solids increases as the length of time of freezing increases."

Openlander and Erb (10) found that condensed skimmilk, when properly frozen and stored, could be used as a satisfactory source of serum solids in ice cream. The first noticeable defect that occurred in the ice cream, made from frozen condensed skimmilk was a curdled appearance on melting. This defect could be easily corrected by the use of 0.3 per cent sodium citrate added to the mix at the pasteurizer. They also found that frozen condensed skimmilk testing 27 to 30 per cent total solids produced an ice cream that was less curdy than ice cream made from frozen condensed skimmilk testing 40 per cent total solids.

Reichert and Corley (15) have recently reported that frozen condensed skimmilk stored at  $-17.8^{\circ}$  C. ( $0^{\circ}$  F.) can be used satisfactorily as a source of serum solids in ice cream manufacture but that a storage period exceeding six months is not advisable. Frozen condensed skimmilk, after storage for four or more months, exhibited evidence of gelation upon melting, indicating that a denaturation of the protein had taken place during storage. However, the appearance of the final ice cream mix was not affected and no trouble was experienced in processing the mix. No consistent differences were noted in the whipping ability of the ice cream mixes nor in the flavor, body, and texture of the finished ice cream, but the ice cream made from the frozen condensed skimmilk melted down approximately twice as fast as that made from fresh condensed skimmilk.

According to Ramsey (13) the proteins in the ice cream mix should be in such a condition that they will absorb moisture readily. He stated that fresh plain condensed skimmilk or superheated condensed skimmilk is the best source of serum solids for ice cream and that sweetened condensed skimmilk and skimmilk powder can be used with fairly good results. Frozen condensed skimmilk is unsatisfactory because the proteins are denatured by freezing.

Tracy (15) and Tracy and Hahn (16) reported that the use of superheated condensed skimmilk in ice cream mixes will likely increase the viscosity of the mix somewhat over that of an ice cream mix containing plain condensed milk. They stated that there is no benefit from the use of superheated condensed skimmilk in ice cream manufacture so far as the time required to reach 100 per cent overrun is concerned. However, when a higher maximum overrun is desired, it may be used to an advantage.

Whitaker and Hilker (18) found that ice cream made from plain condensed skimmilk was less smooth, slightly weaker in body, and had a slightly less cooked flavor than ice cream made from superheated condensed skim-

milk. Using the sales-preference method of comparison, Williams and Hall (19) found that superheated condensed skimmilk produced a better quality of ice cream than did plain condensed skimmilk.

Pyenson and Dahle (12) stated that superheating of condensed skimmilk resulted in increased stability of the proteins, as measured by the alcohol test, and that the change in viscosity obtained by superheating was not due to hydration but to coagulation of the proteins.

#### METHOD OF TREATMENT

*Preparation and Study of the Condensed Skimmilk.* The three types (plain, superheated, and sweetened) of condensed skimmilk used in this study were prepared at three different times of the year (October 12, 1938; December 29, 1938; and February 11, 1939) to increase the scope of the data rather than to observe seasonal variations. The three types of condensed skimmilk prepared at any given period were made from the same lot of raw whole milk and processed in as nearly the same manner as possible.

For the manufacture of the three types of condensed skimmilk, approximately 350 gallons of skimmilk were taken immediately after separation, from pasteurized whole milk. Two-thirds of this skimmilk was heated to a temperature of 150° F. for forewarming and the remaining one-third was held cold, to be used later (approximately four hours) in preparing sweetened condensed skimmilk.

The skimmilk which had been forewarmed to 150° F. was then drawn into a Rogers 26-inch stainless steel vacuum pan and condensed to approximately 30 per cent total solids, under a vacuum of about 25 inches. One-half of the plain condensed skimmilk was drawn from the pan, cooled to about 55° F. a sample taken for analytical purposes and the remainder held at 45° F. until prepared for storage or used in an ice cream mix.

After obtaining about 25 inches of vacuum, the remaining portion of condensed skimmilk in the pan was superheated according to the method used by Doan (4). The temperature of the milk was raised to 180° F. and held for a sufficient length of time (five to fifteen minutes) to cause the desired increase in body as determined by striking and noting thickness. During this time the vacuum dropped from 25 inches to 10 to 13 inches. When the desired thickness was obtained the vacuum pump was started and the superheated condensed skimmilk was cooled in the pan to about 120° F., drawn from the pan and cooled to about 55° F. in a 50-gallon coil vat using ice water as the cooling medium. A sample was taken and the remainder was placed at 45° F. and held until prepared for storage or used in an ice cream mix.

The remaining one-third of the original skimmilk, which had been held cold, was preheated to 150° F. for 20 minutes. An amount of sugar to give one pound of sugar per pound of milk solids was added and the sweetened

skimmilk condensed to approximately 60 per cent total solids (30 per cent milk solids and 30 per cent sugar) under a vacuum of about 25 inches, drawn from the pan and cooled to about 55° F. with a small brine-cooled surface cooler. A sample was taken and the remainder was then placed at 45° F. and held until prepared for storage or used in an ice cream mix.

After the fat and total solids content of the condensed skimmilk had been determined by the Mojonnier procedure, the plain and superheated condensed skimmilk was standardized with distilled water to 30 per cent, total solids. The sweetened condensed skimmilk was not standardized because in all cases the total solids content was slightly less than the desired 60 per cent.

The approximate amount of each type of condensed skimmilk needed for 300 pounds of ice cream mix was weighed into new five-gallon lard tins and stored at approximately 0° F. Three cans of each type of condensed skimmilk were put into storage at each period, one to be taken out at the end of each month for three months and made into an experimental ice cream mix.

As each monthly batch of condensed skimmilk was withdrawn from storage and thawed (at room temperature for 24 hours), observations were made for possible changes in physical structure or presence of "off-flavors" that might have developed during storage. After complete thawing, samples were taken from each type of condensed skimmilk for analytical purposes.

*Preparation of the Ice Cream Mixes.* On the day following the preparation of the condensed skimmilk and at the end of each month of storage three batches of ice cream mix, 300 pounds each, were made, using each of the three types of condensed skimmilk as the source of added serum solids. During this study a total of 35 ice cream mixes was prepared.

Other ingredients used in preparing the ice cream mixes were fresh sweet cream containing 34 per cent butterfat, skimmilk, sugar, corn sugar, and gelatin in quantities to give the following composition: butterfat, 14 per cent; serum solids, 10 per cent; sucrose, 13.5 per cent; corn sugar, 1.5 per cent; gelatin, 0.25 per cent. Each ice cream mix was pasteurized at 160° F. for a period of 20 minutes, homogenized in a two-stage 100-gallon-per-hour Manton Gaulin homogenizer at a total pressure of 3,000 pounds per square inch, with 500 pounds pressure on the second stage, and cooled over a surface cooler to approximately 40° F. and held below 45° F. for 20 to 24 hours.

*Freezing the Ice Cream Mixes.* The ice cream mixes were frozen in a Creamery Package 40-quart horizontal direct-expansion freezer. Before the experimental ice cream mixes were frozen, a preliminary batch was frozen in order to have the freezing conditions as nearly the same as possible. The mixes were frozen in duplicate and in succession, the ice cream mix containing the plain condensed skimmilk always being first, followed in order by the ice cream mixes containing superheated and sweetened con-

densed skimmilk. The temperature of the ice cream mix at the beginning of the freezing process, the temperature of the refrigerant, and the time required to freeze the ice cream mixes to 24° F. were recorded. Overrun readings were taken immediately after the refrigerant was shut off and at minute intervals thereafter until the overrun had reached a maximum.

*Method of Taking Ice Cream Samples.* Samples were obtained by filling quart Sealright containers directly from the freezer as soon as an overrun reading of 100 per cent was obtained, and immediately transferred to the hardening room held at approximately 0° F.

*Analytical Procedure.* The butterfat and total-solids determinations were obtained in duplicate by the Mojonnier method.

Solubility of the condensed skimmilk was determined by standardizing 200 grams to 9 per cent milk solids with distilled water at 70° F., mixing for one minute on an electric Hamilton-Beach Malted Milk Mixer and centrifuging a 25 ml. portion for 15 minutes in a Hart Casein test bottle at normal Babcock speed. The supernatant liquid was removed by means of a rubber siphon tube to within 2 or 3 ml. of the sediment and the test bottle refilled to the 25 ml. mark with distilled water. The test bottles were then shaken to dislodge the sediment in the bottom of the bottle and again centrifuged for 15 minutes. Each sample was represented by duplicate tests and the degree of solubility rated relatively as the number of smallest graduations on the Hart Casein test bottle, according to the amount of sediment collected in the bottom of the test bottle.

Protein stability of the ice cream mixes was determined by the alcohol number method used by Pyenson and Dahle (11). The titratable-acidity of the ice cream mixes was obtained by weighing nine grams of the sample at room temperature into a 125 ml. Erlenmeyer flask, adding 9 ml. of boiled distilled water, five drops of a one per cent alcoholic solution of phenolphthalein and titrating to the first definite and relatively permanent shade of pink with N/10 sodium hydroxide.

The pH of the ice cream mixes was determined at 25° C. (77° F.) by means of a quinhydrone electrode and a Leeds and Northrup Type K potentiometer.

Viscosity of the ice cream mixes was measured in triplicate at 5° C. (41° F.) with a Gramercy model MacMichael viscometer, using a 100 ml. sample and a No. 30 wire. The average of the three determinations was recorded as the viscosity in MacMichael degrees.

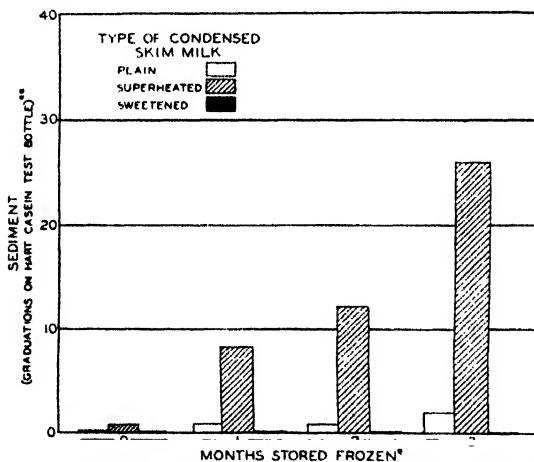
*Judging the Ice Cream and Determining Melting Quality.* The quart samples of ice cream were removed from the hardening room about 10 days after freezing and were cut in half. One pint of each sample was scored for flavor, body, and texture, and the remaining pints were set upon one-fourth-inch-mesh wire, resting on the rim of a heavy five-inch glass funnel with the end leading into a previously weighed 100 ml. graduated cylinder.

The number of minutes required for 100 ml. of melted ice cream to collect in the cylinder in a room held at 60° to 62° F. were recorded. The character of the melted ice cream as to smoothness and amount of foam was observed.

#### EXPERIMENTAL RESULTS

*Physical Appearance of the Three Types of Condensed Skimmilk as Affected by Freezing and Storing.* Comparisons were made concerning the body and physical appearance of each type of fresh condensed skimmilk and again at the end of each storage period (table 1). The superheated condensed skimmilk was changed most in physical appearance as shown by the slight curdy appearance and whey separation at the end of one month in storage, increased protein precipitation after two months, and marked precipitation after three months. The plain condensed skimmilk, which had been prepared in October, evidenced slight whey separation after two months in storage but was not criticized otherwise during this study. Since this defect did not occur in the plain condensed milk prepared in December and February, the defect may be ascribed to the quality of the original milk from which the October batch was prepared. Except for a slight sandy condition, there was no change in the physical appearance of the sweetened condensed skimmilk after three months in storage.

Fresh plain, and sweetened condensed skimmilk were almost completely



GRAPH 1. Comparative solubility of condensed skim milks. Fresh and stored frozen for the periods of time indicated.

\* Average data from 3 series with the exception of 3 month period which is an average of only 2 series.

\*\* Solubility is expressed as the number of smallest graduations on the graduated scale of the Hart casein test bottle.

soluble, and but very slightly more soluble than the fresh superheated condensed skimmilk. After one month in storage, the solubility of the plain condensed skimmilk decreased and continued to decrease very slowly up to the end of three months in storage. The solubility of the superheated condensed skimmilk decreased rapidly during one month in storage and continued to decrease rapidly during the storage period. The sweetened condensed skimmilk was almost completely soluble after three months in storage.

TABLE 1

*Observations on the appearance of the condensed skimmilk, fresh and after thawing*

Type of condensed skimmilk	Age (months frozen)	Date condensed		
		Oct. 12, 1938	Dec 29, 1938	Feb. 11, 1939
Plain	0 (Flesh)	good	good	good
	1	sandy	good	good
	2	sandy	good	good
	3	sl. whey	good	good
Superheated	0 (Fresh)	good	good	good
	1	sl. whey	sl. whey	sl. whey
	2	sl. curdy	sl. curdy	sl. curdy
	3	whey, curdy sl. gel.	sl. whey sl. curdy curdy, gel., whey	sl. whey curdy sl. whey curdy
Sweetened	0 (Fresh)	good	good	good
	1	sl. sandy	sl. sandy	good
	2	sl. sandy	sl. sandy	good
	3	sl. sandy	sl. sandy	good

*Chemical and Physical Characteristics of the Ice Cream Mixes as Affected by the Three Types of Fresh and Stored Frozen Condensed Skimmilk Protein Stability.* There was no consistent change in the protein stability (alcohol number) of ice cream mixes made with condensed skimmilk which had been stored frozen one month when compared to those prepared with the fresh product (table 2). However, mixes made with stored frozen superheated condensed skimmilk at this period showed less change than mixes made with plain or sweetened condensed skimmilk. It was also noticed that the protein stability of ice cream mixes, made with each type of condensed skimmilk which had been stored frozen two months, was consistently greater than that of mixes made with fresh condensed skimmilk or condensed skimmilk which had been stored frozen for one and three months. There was no apparent relationship between the solubility of the condensed skimmilk and the alcohol number of the ice cream mix in which it was used.

*Titrable Acidity.* There was very little difference in the titrable acidity of the ice cream mixes made with the three types of fresh condensed skim-

TABLE 2  
*Comparison of some chemical and physical properties of ice cream mixes, made from fresh and stored frozen condensed skim milk*

Condensed skimmilk										Ice cream mix					
Type and age	Solubility			Total solids			Butterfat			Viscosity†			Alcohol No.‡		
	I*	II*	III*	I*	II*	III*	I*	II*	III*	I*	II*	III*	I*	II*	III*
Plain				%	%	%	%	%	%						
Fresh	0	0	0	38.96	39.14	39.13	14.01	14.02	13.99	53	110	67	5.5	5.5	5.5
1 mos.	1	1	0	39.61	39.21	39.27	14.24	14.15	14.01	69	92	79	5.0	5.5	6.0
2 mos.	1	1	0	39.24	39.34	39.31	13.95	14.12	14.12	98	93	73	6.0	7.0	6.5
3 mos.	...	2	2	...	39.19	39.38	13.99	14.07	14.07	...	67	92	...	6.0	5.5
Superheated															
Fresh	0	1	2	38.96	39.19	39.35	14.00	14.01	14.01	49	98	114	5.5	5.5	5.5
1 mos.	11	8	16	39.55	39.24	39.11	14.23	14.20	14.00	104	103	125	5.5	5.5	5.5
2 mos.	10	10	32	39.32	39.14	39.43	14.02	14.04	14.09	104	82	101	5.5	7.0	6.5
3 mos.	...	20	32	...	39.38	39.40	...	14.14	14.11	...	203	84	...	6.0	5.5
Sweetened															
Fresh	0	0	0	39.31	39.46	39.21	14.02	14.16	14.02	53	101	86	6.0	6.0	3.5
1 mos.	0	0	0	39.31	39.17	39.01	14.05	14.21	13.98	112	75	91	5.0	5.5	5.0
2 mos.	0	0	0	39.44	39.42	39.40	14.28	14.15	14.12	118	67	63	6.0	6.0	6.5
3 mos.	0	0	0	38.95	39.37	39.37	...	14.02	14.02	...	78	65	...	5.5	5.5

\* Indicates date of making the condensed milk: I—Oct. 12, 1938; II—Dec. 29, 1938; III—Feb. 1, 1939.

† Viscosity is expressed in MacMichael degrees.

‡ Alcohol number is the ml. of 95% alcohol required to give the first sign of precipitation in a 5 ml. sample.

milk (table 3). The titratable acidity of the ice cream mixes made with the three types of stored frozen condensed skimmilk was in nearly every case equal to or less than the titratable acidity of mixes made with the fresh condensed skimmilk, thus indicating that freezing and storing condensed skimmilk for periods up to three months did not increase the titratable acidity of the ice cream mix in which it was used.

*Effect on pH.* The pH of ice cream mixes made with fresh sweetened condensed skimmilk was in every case slightly lower than the pH of ice cream mixes made with fresh plain and fresh superheated condensed skimmilk. This variation might be explained in part by the fact that even though the three types of condensed skimmilk were prepared from the same original batch of raw milk, the skimmilk used in preparing the sweetened condensed skimmilk was always subjected to a holding period of approximately four hours at 45° F. before it was drawn into the condensing pan. The pH of the ice cream mixes was always within the range of 6.2 and 6.4 and the differences were not significant as far as the type of fresh and stored frozen condensed skimmilk is concerned.

*Effect on Viscosity.* Viscosity determinations show (table 2) that ice cream mixes made with superheated condensed skimmilk were but slightly more viscous than ice cream mixes made with plain and sweetened condensed skimmilk, indicating that the high viscosity of the superheated condensed skimmilk did not carry over appreciably into the ice cream mix. The viscosity of the ice cream mixes was not consistently affected by the freezing and storing of the three types of condensed skimmilk used.

*Whipping Ability.* Conclusions drawn from average whipping curves indicate that the ice cream mixes made with the fresh plain condensed skimmilk whipped to 100 per cent overrun faster than ice cream mixes made with fresh superheated and fresh sweetened condensed skimmilk. There was no significant difference in the time required to reach 100 per cent overrun in mixes made with the latter two types of condensed skimmilk, but a higher maximum overrun was obtained in those mixes made with fresh sweetened condensed skimmilk. There was an increase in time required to reach 100 per cent overrun in mixes made with the three types of condensed skimmilk stored frozen one month, and the maximum overrun was lowest. The time required to reach 100 per cent overrun in ice cream mixes made with the three types of condensed skimmilk stored frozen for two and three months was less than the time required for mixes made with fresh condensed skimmilk and the maximum overrun was approximately the same as that of ice cream made with fresh condensed skimmilk.

*Flavor, Body, and Texture and Melting Characteristics of the Ice Cream.* In general there was no appreciable difference in the flavor scores of ice cream which could be attributed to the type of condensed skimmilk used

TABLE 3  
*Comparison of some chemical and physical properties of ice cream mixes, made from fresh and stored frozen condensed skimmilk*

Condensed skimmilk		Ice cream mix													
		Titratable acidity			pH			Time to reach 100% overrun			Time to reach max. overrun			Maximum overrun	
Type and age	I*	II*	III*	I*	II*	III*	I*	II*	III*	I*	II*	III*	I*	II*	III*
Plain	%	%	%				min.	min.	min.	min.	min.	min.	%	%	%
Fresh	.200	.188	.196	6.41	6.33	6.39	5.0	8.5	9.5	14	20	17	133	144	129
1 mo.	.200	.196	.206	6.39	6.29	6.39	7.0	11.0	8.0	15	19	13	133	132	126
2 mos.	.179	.196	.169	6.36	6.40	6.40	8.0	6.5	6.5	18	14	126	146	120	146
3 mos.	...	.183	.183	...	6.38	6.39	...	6.0	4.0	...	16	17	...	136	142
Superheated															
Fresh	.200	.188	.196	6.39	6.33	6.39	6.5	9.0	9.0	15	15	18	123	134	132
1 mo.	.188	.188	.186	6.37	6.30	6.41	9.0	11.0	9.0	16	20	16	119	127	130
2 mos.	.179	.196	.173	6.23	6.40	6.36	7.5	9.0	7.0	13	14	16	124	141	125
3 mos.	...	.178	.173	...	6.39	6.40	...	11.0	6.0	...	20	19	...	121	133
Sweetened															
Fresh	.200	.194	.227	6.38	6.32	6.27	7.0	9.5	8.0	14	20	20	125	144	135
1 mo.	.183	.188	.206	6.38	6.29	6.39	8.0	11.0	8.5	15	20	16	133	127	124
2 mos.	.179	.196	.183	6.36	6.40	6.40	8.0	8.5	6.5	16	16	17	133	141	127
3 mos.	...	.183	.183	...	6.40	6.39	...	7.5	6.0	...	17	19	...	130	136

\* Indicates date of making the condensed milk: I—October 12, 1938; II—December 29, 1938; III—February 1, 1939.

or whether it was the fresh or frozen product. From the standpoint of body and texture, there was no difference in ice creams made with the three types of fresh condensed skimmilk. However, there was a tendency for the ice creams made with the three types of condensed skimmilk stored for one month to score slightly lower in body and texture than that made with fresh condensed skimmilk.

Ice cream made with fresh superheated condensed skimmilk was slower in melting than ice cream made with either fresh plain or sweetened condensed skimmilk. Freezing and storing superheated condensed skimmilk had no consistent effect on the melting time of the ice cream. Ice cream made with fresh sweetened condensed skimmilk melted faster than ice cream made with fresh plain condensed skimmilk, but ice cream made with the same condensed skimmilk after one, two, and three months in storage showed exactly opposite results. The melting time for the ice cream made with plain condensed skimmilk stored one month did not change, whereas that of ice cream made with sweetened condensed skimmilk during the same period increased. These data indicate that freezing and storing plain condensed skimmilk brought about a change that did not occur in the sweetened condensed skimmilk. These differences, however, were not great and would not suggest an advantage of one type of condensed skimmilk over the other.

As the melting time decreased the weight of the melted ice cream also decreased. By studying the weight of the melted ice cream, the amount of foam could be relatively determined. Of the three types of condensed skimmilk used in the ice cream, the fresh sweetened condensed skimmilk gave the most foam on melting, but after the condensed had been in storage for one month, ice cream made with the plain condensed skimmilk gave the most foam. Ice cream made with superheated condensed skimmilk always gave the least amount of foam on melting. Freezing and storing the plain and superheated condensed skimmilk increased the amount of foam in the melted ice cream, whereas the opposite was true for sweetened condensed skimmilk.

Ice cream made with plain condensed skimmilk melted down smoothly and evenly in every case. The ice cream made with stored frozen superheated condensed skimmilk was frequently criticized for a slight curdy appearance, but the most noticeable difference in melting of this type of ice cream was that, instead of the sides melting straight down as they did in the ice cream made with plain and sweetened condensed skimmilk, they tended to fall off in chunks. Ice cream made with fresh sweetened condensed skimmilk always melted smoothly and evenly, but that made with stored frozen sweetened condensed skimmilk was sometimes criticised for having a flaky or curdy appearance.

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# THE EFFECT OF ADDED EGG PHOSPHOLIPIDS ON THE NUTRITIVE VALUE OF CERTAIN VEGETABLE OILS<sup>1</sup>

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It was recently reported from this laboratory (1) that butter fat has a higher nutritive value for growth in weanling rats than certain vegetable oils when homogenized into mineralized skimmed milk and supplemented with all the known essential fat soluble vitamins. The difference in the nutritive value was not found to be due to factors contained in the non-saponifiable fraction of butter fat (1). However, since the phospholipids are decomposed upon saponification it appeared possible that the difference in the nutritive value of butter fat and the vegetable oils fed might be due to some particular phospholipid contained in the butter fat which was not contained in the vegetable oils. The work described in this paper was carried out to determine if a common available phospholipid, such as egg phosphatide, or the nitrogenous bases of phosphatides, had any effect on the nutritive value of the vegetable oils used in our experiments.

## EXPERIMENTAL

Weanling rats weighing between 30 and 35 grams were used. Six rats, 3 males and 3 females, were placed on each fat in each particular experiment as described in a previous paper (1). The fats were homogenized into skimmed milk at a level of 4 per cent and fed ad libitum. Twenty micrograms of carotene were added to each gram of fat, 100 micrograms of  $\alpha$ -tocopherol<sup>2</sup> were given to each rat every week and all animals were irradiated 10 minutes daily. The milks were mineralized with iron, copper, and manganese so that each 100 cc. contained 1.5 mg. of iron, 0.15 mg. of copper, and 0.15 mg. of manganese.

The phosphorus content of the oils used in these experiments was found to be approximately 10 mg. per 100 grams of butter fat, 3 mg. per 100 grams of corn oil, and 8 mg. per 100 grams of coconut oil. From the phosphorus analysis, if all the phosphorus was in the oils as phospholipids containing 4 per cent of phosphorus, the phospholipid content of butter

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should be 0.25 per cent, corn oil 0.075 per cent, and coconut oil 0.2 per cent. The phospholipid content of skimmed milk has been reported to be 0.015 per cent and of butter 0.2 per cent (2). The skimmed milk used for making up milks should have supplied about the same amount of phospholipids as the fat, but with corn oil the amount of phospholipid consumed would be considerably lower than with butter fat or coconut oil. However, soy bean oil, which was inferior to butter fat for growth in young rats (1), was found to contain as much as 120 mg. phosphorus per 100 grams, or 3 per cent phospholipids, on the basis of 4 per cent phosphorus in the phospholipids. On the basis of these results it was considered best to feed one of the common available phospholipids (egg lecithin) along with corn oil and coconut oil.

These experiments were made with egg lecithin (Pfanstiehl Pure Ex-Ovo-soluble) added to corn oil and coconut oil at a level of 0.25 per cent. Comparisons were also made with butter fat and the vegetable oils without lecithin. Some response was obtained on the oils with egg lecithin added to the oils alone but the growth was not as good as the growth obtained on butter fat. The addition of lecithin seemed to improve the appearance of the hair coat of the animals considerably.

Since some response was obtained with 0.25 per cent lecithin in the vegetable oils, a higher level was tried. In a second trial with the same experimental set up but with 0.5 per cent of egg lecithin added to the oils, no better response was obtained than was found in the first trial. A third experiment with the lecithin content of the oils at 0.5 per cent resulted in a poorer response with considerable variation. Figure 1 illustrates the

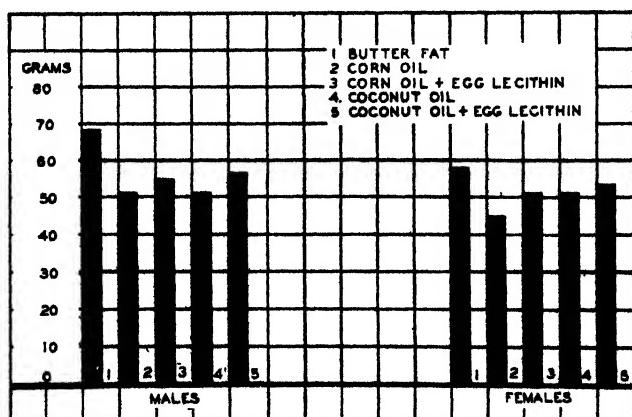


FIG. 1. The bars indicate average gains made during the first three weeks of the experiment—representing 18 rats, 9 males and 9 females, on each fat.

average weight gains made for these trials during the first three weeks on the experiment with the various oils.

Another experiment was set up comparing butter fat, corn oil, corn oil plus sphingomyelin,<sup>3</sup> corn oil plus sphingosine,<sup>3</sup> corn oil plus ethanolamine, and corn oil plus choline. The phosphatides and bases were suspended or dissolved in water and fed in the milk each day. The following amounts were fed to each rat on the particular diet per day. Sphingomyelin 0.6 mg., sphingosine sulfate 0.5 mg., ethanolamine 1 mg., and choline 1 mg. Limited sources of sphingomyelin and sphingosine sulfate prevented these substances being fed at higher levels. No particular differences were observed in weight and appearance between the animals on corn oil alone and corn oil plus the phosphatide bases except with the females on choline. However, this group of females did not do as well as the animals on butter fat. Figure 2 illustrates the average gain in weight during the first three weeks of the experiment.

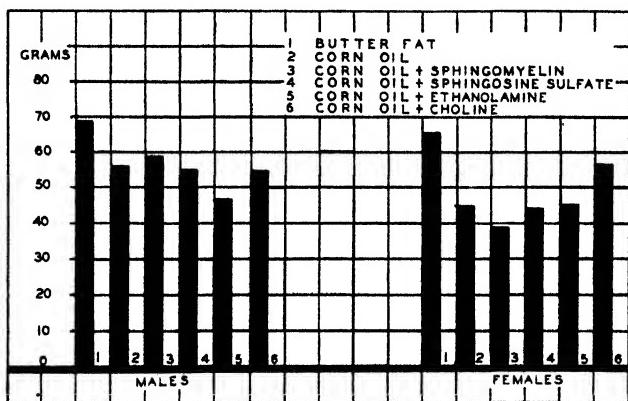


FIG. 2. Bars represent average gains made during the first three weeks of the experiment with phosphatide bases added to corn oil.

#### DISCUSSION

It appears from the results obtained that egg lecithin improved the nutritive value of corn oil and coconut oil slightly but not enough to give growth equal to that obtained on butter fat. In certain cases individuals on the vegetable oils plus lecithin did as well as those on butter fat but in most instances the growth was inferior to animals on butter fat. Sphingomyelin, sphingosine sulfate, and ethanolamine did not improve the nutritive value of corn oil while choline seemed to improve corn oil slightly. Since in a previous experiment (1) soy bean oil was found to be inferior

<sup>3</sup> We are very grateful to Dr. P. A. Levene, Rockefeller Institute for Medical Research, for the sphingosine and sphingomyelin used in these experiments.

to butter fat irrespective of its high phospholipid content, it appears that something besides the phospholipid is responsible for the superior growth obtained on butter fat with weanling rats.

#### CONCLUSIONS

1. Addition of 0.25 per cent and 0.5 per cent of egg lecithin to corn oil or coconut oil improved the nutritive value of these oils slightly but not enough to make them equal to butter fat when they were homogenized into mineralized skimmed milk at a level of 4 per cent and fed to weanling rats.

2. Sphingomyelin, sphingosine sulfate, and ethanolamine had no effect on the nutritive value of corn oil, but choline, in the case of the females, seemed to improve it slightly.

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## THE NUTRITIVE VALUE OF THE FATTY ACID FRACTIONS OF BUTTER FAT<sup>1</sup>

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In a previous paper (1) it was reported that butter fat homogenized into mineralized skim milk gave better growth of weanling rats than certain vegetable oils homogenized into skim milk and fed under the same conditions with ample carotene, irradiation,  $\alpha$ -tocopherol and minerals added in all cases. The superiority of butter fat for growth was not found to be due to factors contained in the non-saponifiable fraction of butter or to be due to compounds such as lecithin, choline, sphingomyelin or sphingosine (2). It was then thought advisable to separate the fatty acids of butter into various fractions and feed the glycerol esters of these fractions along with a vegetable oil in concentrations approximately equal to that found in butter fat.

### EXPERIMENTAL

The fatty acid fractions of the butter fat were prepared as follows: Five hundred grams of melted butter fat were poured into 1000 cc. of 20 per cent alcoholic potassium hydroxide solution and heated on the steam bath for about one-half hour. The alcohol was evaporated off in an open dish, the soaps dissolved in water and the separations made similar to a procedure described by Hilditch and Jones (3). The solution was neutralized with sulfuric acid and steam distilled until about 5 liters of distillate were collected. The volatile acids were extracted from the distillate by 5 successive extractions with ether. The non-volatile fraction was washed thoroughly with hot water and dissolved in 2000 cc. of alcohol. The alcohol solution was heated almost to boiling and 200 gm. of lead acetate crystals added. The solution was allowed to cool and the insoluble lead soaps filtered off and recrystallized from alcohol. After removing, by vacuum distillation, the alcohol from the soluble lead soaps, which constitutes most of the unsaturated fatty acids, the material was washed with hot water and the free fatty acids regenerated by adding dilute sulfuric acid until the solution was distinctly acid to congo red. The acids were separated from the water and precipitated lead sulfate, washed with hot water and taken up in ether to remove the last traces of lead sulfate. The insoluble lead soaps which constitute most of the saturated acids but still contaminated

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with some of the unsaturated acids were treated in exactly the same manner as the unsaturated acids and finally taken up in ether to remove the last traces of lead sulfate. The ether was removed from the various fractions under vacuum. Five hundred gm. of butter fat yielded 36.5 gm. of volatile acids, 180 gm. of the unsaturated acid fraction and 200 gm. of the saturated fraction.

The triglycerides were synthesized as follows (4). The fatty acids from each fraction were placed in a suitable round bottom distilling flask and the theoretical amount of glycerol added as calculated from the fatty acid composition of each fraction (3). The flask containing the fatty acids and glycerol was placed in an oil bath and heated to 200° C. for six hours with a fine stream of carbon dioxide passing through the mixture. The glycerides of each fraction were then mixed with corn oil (Mazola) in the following proportions:

Triglycerides of volatile acids	7 parts,	corn oil	93 parts
" " unsaturated "	40 "	" "	60 "
" " saturated "	55 "	" "	45 "

These are approximately the proportions of the different fractions isolated from the butter.

Weanling rats about 20 days old and weighing 30 to 35 gm. were used. Six rats, 3 males and 3 females, were placed on each fraction to be tested and kept in individual cages. Five groups were set up and fed as follows, ad libitum:

Group	I.	Butter fat milk
"	II.	Corn oil milk
"	III.	Corn oil plus volatile fraction milk
"	IV.	" " unsaturated " "
"	V.	" " saturated " "

The fats were homogenized into fresh skim milk with a small hand homogenizer and all milks were made up to contain 4 per cent fat. Twenty micrograms of carotene were added to each gram of fat and 100 micrograms of  $\alpha$ -tocopherol acetate<sup>2</sup> were given to each rat every week. All rats were irradiated 10 minutes each day. All milks were mineralized with iron, copper and manganese so that each 100 cc. of milk contained 1.5 mg. of iron, 0.15 mg. copper and 0.15 mg. of manganese.

The results obtained are illustrated in figures 1 and 2 (Experiment 17) and figures 3 and 4 (Experiment 19). The animals on corn oil plus the saturated fraction of butter fat (Group V) grew faster than the animals on butter fat and considerably faster than the animals on corn oil, or on the volatile or unsaturated fractions. The data in figures 1 and 2 do not

<sup>2</sup> We are indebted to Hoffmann LaRoche, Inc., Nutley, New Jersey, for generous supplies of dl- $\alpha$ -tocopherol acetate.

include the unsaturated fraction because shortly after mixing the tri-glycerides with corn oil the mixture began to thicken and develop an odor somewhat like paint. The animals on this fraction failed to grow. In the second trial, Experiment 19, figures 3 and 4, the fractions from butter were

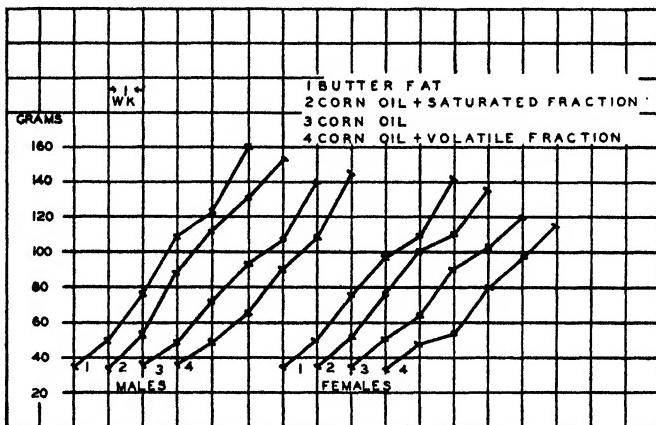


FIG. 1. Experiment 17. Curves showing average weights for each week for rats on milks made up with butter fat, corn oil and fatty acid fractions of butter fat representing 6 rats, 3 males and 3 females, on each fat.

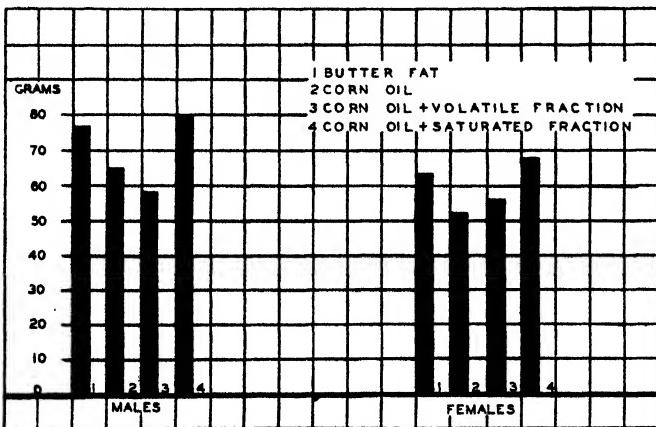


FIG. 2. Experiment 17. Bars representing gain made during the first three weeks on experiment representing 6 rats, 3 males and 3 females, on each fat.

mixed with corn oil just before being homogenized into the milk. The males on butter fat in Experiment 17, figure 2, made an average gain of 76 gm., on corn oil 65 gm., on the volatile fraction 57 gm., and on the saturated fraction 80 gm. in three weeks. The females on butter fat in this experiment made an average gain of 63 gm., on corn oil 52 gm., on the vola-

tile fraction 56 gm., and on the saturated fraction 68 gm. in 3 weeks. The males on butter fat in Experiment 19, figure 4, made an average gain of 70 gm., on corn oil 63 gm., on the volatile fraction 48 gm., on the unsaturated fraction 63 gm., and on the saturated fraction 82 gm. gain in 3 weeks. The

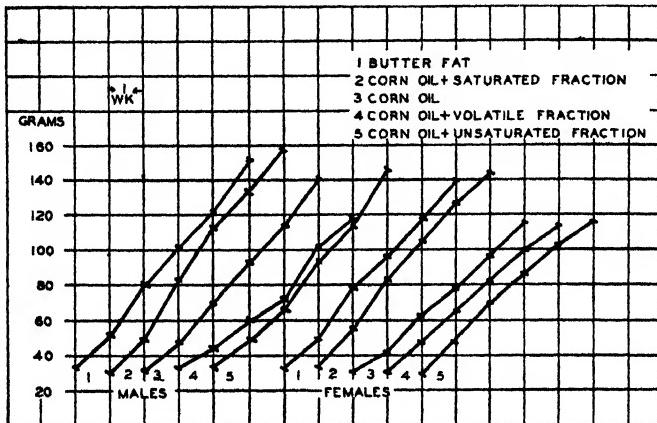


FIG. 3. Experiment 19. Curves showing average weights for each week for rats on milks made up with butter fat, corn oil, and fatty acid fractions of butter fat representing 6 rats, 3 males and 3 females, on each fat.

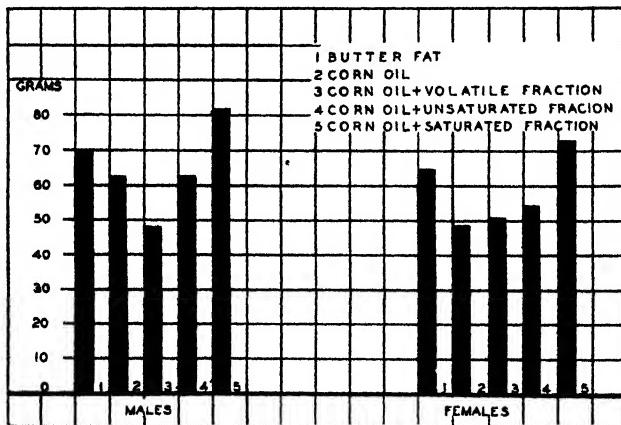


FIG. 4. Experiment 19. Bars representing gain made during the first three weeks on experiment representing 6 rats, 3 males and 3 females, on each fat.

females on butter fat gained an average of 66 gm., on corn oil 47 gm., on the volatile fraction 51 gm., on the unsaturated fraction 55 gm., and on the saturated fraction 73 gm. in 3 weeks. The general appearance and condition of the fur coat of the animals on the saturated fraction was better during the first two or three weeks on the experiment than the other groups.

However, after this time there was very little difference between the groups in appearance.

Food consumption records indicate that the animals on the saturated fraction did not make more efficient gains than animals on butter. That is, growth paralleled consumption very closely. However, we have found that the faster-growing animals usually made slightly more efficient gains than animals growing at a slower rate and these differences appeared between groups of rats on butter fat and vegetable oils during the first two or three weeks of the experiment.

#### DISCUSSION

The results obtained indicate that the superiority of butter fat fed in the milks is due to fatty acids contained in the *saturated* fraction of butter fat. It also appears that the superiority is due to long chain saturated fatty acids. The saturated fraction was found to have an iodine number of 9.2, showing that some unsaturated fatty acids still remained. However, if the superiority of growth was due to unsaturated fatty acids then it appears that corn oil or the unsaturated fraction of butter fat should have supplied the necessary fatty acids. Hilditch and Jones (3) found the saturated fatty acid fraction of butter fats to have an iodine number of 10 and believed that the unsaturated fatty acids in the saturated fraction were only those carried over in the separation. However, no final proof can be given that an unsaturated fatty acid, being insoluble as the lead soap, is not responsible for the superiority until the saturated fraction is completely hydrogenated and fed.

No final explanation can be offered for the observation that better growth was obtained with the saturated butter fraction and corn oil as compared with butter alone. It is probable that the fatty acids which are responsible for the superiority of butter over vegetable oils are present in larger amounts in butter fat, and in the mixture of corn oil and the saturated fraction from butter fat an increased amount of saturated fatty acids of high molecular weight made possible the accelerated growth.

#### CONCLUSIONS

1. The fatty acids responsible for the superior growth of young rats obtained on butter fat as compared with certain vegetable oils homogenized into skim milk with all of the known essential fat soluble vitamins added, apparently lie in the saturated fraction of butter fat.
2. When the fatty acids of butter fat were separated into the volatile acids by steam distillation and into the unsaturated and saturated acids as lead soaps and the triglycerides of these fractions fed in corn oil in approximately the composition found in butter the saturated fraction with

corn oil was found to be a little superior to butter fat while the other two fractions compared favorably with corn oil.

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THE LENGTH OF THE INTESTINE OF CALVES AND ITS  
BEARING ON THE ABSORPTION OF THE  
NUTRIENTS FROM THE CHYME<sup>1</sup>

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In his recent book (1) Alvarez states, "Meat-eating animals have a simple stomach and colon and a short bowel, whereas grass-eating animals usually have a complicated stomach and colon, a large cecal pouch, and a long, small bowel. In carnivorous animals the small bowel is said to be only from four to eight times the body length, while in herbivorous animals it is from twenty-five to seventy-five times the body length." According to Swett and Graves (15) the length of the small intestine of mature beef cows (eight animals) varies from 93 to 140 feet and that of dairy cows (two animals) from 144 to 172 feet. From these data one finds that the post-mortem length of the small intestine of beef and dairy cattle is 28 and 33 times, respectively, the length from withers to pin bones, or within the lower limit suggested by Alvarez. The length of the large intestine of the same animals varies from 23 to 41 feet for beef animals and from 43 to 46 feet for dairy animals.

Black, Semple and Lush (2) found that the average length of the small intestine of 20 seven-months old range steers was 98 feet. One hundred and twenty days later the average length of the small intestine from a similarly bred group (32 animals) had increased to 110 feet. The average length of the large intestine for both groups was 21 feet. Such figures, of course, are only approximate in that the changing tone of the musculature of the intestine makes an exact figure impossible.

Data concerning the length of the intestine of a live calf has only been reported on one animal (6). In this instance the length of the small intestine of a six months old calf was found to be 21 feet two inches in the living animal and 68 feet nine inches on removal from the body. Since that time the following data shown in table 1 have been accumulated:

From these data it is apparent that the length of the small intestine of the living calf is about seven times its body length, a figure somewhat out of line with that suggested by Alvarez.

What influence this greater length of the small bowel has on digestion can be better understood by studying the movement of chyme in the gastrointestinal tracts of carnivora and herbivora. Heile (10) states that in dogs on a mixed meal, material began to appear at the lower end of the

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TABLE I  
*Length of small and large intestine of calves in vivo and post mortem*

Calf number	Age in mo.	Length of small intestine				Length of large intestine			
		In vivo		Post mortem		In vivo		Post mortem	
		Ft.	In.	Ft.	In.	Ft.	In.	Ft.	In.
47A4	5	23	3	77	1				
47R	9	20	1	53	4	7	6*	12	10*
1698	12	17	2	68	10	7	1	14	8
1704	10	25	5	81	1	7	2	16	10
Previously reported (6)	re- ported (6)	21	1	68	9				
Average		21	5	69	10	7	2	15	9

\* Data do not include cecum. Consequently figures have not been used in calculating average.

small bowel one hour after feeding and by the fifth hour the small bowel was empty. With a diet rich in carbohydrates and fats, the time required for digestion and elimination from the stomach and small intestine was only three to four hours. Assuming the usual time for digestion in the stomach of dogs, it is apparent that the absorption of the digestible portion of the meal and the elimination of the residue into the large gut occurs within an hour after the food enters the small intestine. In other words, if the average length of the small bowel is about four feet (1, 3) the chyme must be moving through this part of the intestinal tract at an average rate of at least four feet per hour.

Comparable data for ruminants are not available. Dickey (4) found that the first part of a meal of milk passed through the entire gastrointestinal tract of young calves in 30 hours while Fish (8) reported that the first of a dye marked feed offered to mature cattle passed through the gastrointestinal tract in 16 to 17 hours. The time required for the passage of all the food residues from one meal through the digestive tract of herbivora may require several days (5). Ewing and Smith (7) claim that during most of this time the food is in the rumen. It is entirely probable that the first residues to reach the anus from any one meal are from food which has not been detained long in any part of the stomach. The average time for the major portion of the food to pass through the gastrointestinal tract of mature herbivora probably is about 72 to 84 hours, depending on the amount and type of feed, its physical condition, and certain physiological reactions in the animals (7). What then is the rate at which the food residues pass through the various segments of the intestine?

Available information would indicate that food nutrients other than the fiber fraction are equally well absorbed by ruminants and non-ruminants despite a difference in the length of their respective intestinal tracts. Due to the physical effect of the fiber the progress of food through the small

intestine of herbivora is probably faster than for carnivora. In fact, this would seem logical from two unrelated bits of evidence. First, Krzywanek (12) and others (9, 11, 16) found that the rate of travel of the ingesta is speeded up by giving food at frequent intervals. The act of filling the stomach also increases the motility of the colon, frequently to the point of causing immediate defecation. With ruminants it is a well known fact that food from the first two compartments of the stomach is emptied into the abomasum at frequent intervals. This frequent stimulus may aid in the more rapid progress of the chyme through the bowel of the ruminant as compared to that of the non-ruminant.

A factor of even more importance in increasing the rate of passage of food through the bowel is the fiber content. Foods rich in cellulose tend to pass through the small bowel faster than low fiber feeds (13, 14). This in itself may explain why a ruminant needs a longer small intestine, relatively, than a dog in order to utilize its feed efficiently.

It has been the experience of the investigators that high fiber material, as used in most "milk substitutes," tends to cause young calves to scour. Unground or coarsely cut roughage does not have this effect, probably because it is held in the rumen until partially "digested." This predisposition to scour on ground high fiber feeds vanishes as soon as the first three compartments of the stomach have reached sufficient size to hold back one or two days' food supply until acted upon by bacteria and the rumen fluid. Apparently, the intestine of the young calf is quite sensitive to large amounts of fiber. Although the small intestine of the ruminant is two to three times as long, relatively, as that of the non-ruminant, this additional length is not sufficient to provide for the efficient use of high fiber feeds unless thoroughly comminuted in the rumen and passed into the abomasum in small amounts. Grinding of the feed cannot replace this action of the rumen.

The large intestine of the ruminant is relatively short. In that its length is comparable to that of the large bowel in carnivora, there is no reason for believing that it plays an active part in the utilization of a high fiber feed.

#### SUMMARY

The small intestine of the living calf is about seven times the body length, or about one-third the post-mortem length. Variations in the ratio between body length and length of intestine depend more on individuality than upon the age of the calf. Although the progress of the chyme through this region is comparatively rapid, the increased length in ruminants allows for proper absorption of the nutrients if thoroughly comminuted in the rumen.

The large intestine does not show as great a difference in length between the living and post-mortem stages as does the small intestine.

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## EFFECT OF SALT ON THE KEEPING QUALITY OF CREAM<sup>1</sup>

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### INTRODUCTION

The problem of preventing deterioration in cream from the time it is produced until it is finally made into butter is of vital concern to the creamery industry. In many cases the limited volume of cream produced per farm may not justify the expense involved in providing adequate facilities for cooling and storage; and daily or even frequent deliveries of the cream cannot be expected. Before delivery to the cream station or creamery, much of the cream used in the manufacture of butter has been held for several days at temperatures favorable for rapid growth of the microorganisms which cause deterioration.

The greatest need in improving the quality of cream and butter produced throughout much of the Middlewest is in finding some inexpensive, simple procedure which could be used by the producer to prevent or retard cream deterioration. Williams (1) of the United States Department of Agriculture patented a method of preserving cream by the addition of salt. This patent (U. S. patent No. 2,192,864 granted in January, 1939) has been dedicated to the free use of the people of the United States. The use of this method, however, has not been legalized by the United States Food and Drug Administration, which contends that cream to which salt has been added is an adulterated food product.

This investigation was undertaken to determine the merits and the limitations of improving the keeping quality of cream by the addition of salt. The principal objectives of this study were:

1. To determine the effectiveness of various concentrations of salt in retarding or preventing deterioration of sweet cream stored at various temperatures.
2. To determine the effectiveness of salt in stopping deterioration in cream stored at 70° F. for various intervals before the salt was added.
3. To determine the effect of salt in cream on the quality of the resulting butter.
4. To determine the effect of salt in cream on the accuracy of the Babcock test.

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## REVIEW OF LITERATURE

Williams (1) claimed that cream to which about seven per cent salt had been added could be held for one week at room temperatures without appreciable deterioration, and such cream could be used in the manufacture of sweet cream butter; or if reseparated into a plastic cream, it could be used as a substitute for sweet market cream.

Thompson and Macy (2) indicated that the addition of 7.5 to 10 per cent salt to cream stored for 10 days at temperatures of 70° F. or lower, effectively retarded bacterial growth and acid development, and prevented the cream from developing off-flavors except for a slight staleness. Control lots of the same cream held without salt were criticized for such defects as "cheesy," "yeasty" or "alcoholic."

Castell and Garrard (3) reported that sweet fresh cream containing 7 per cent salt could be held in a satisfactory condition for eight days at 77° F. They observed that oxidizing bacteria, believed to be responsible for cheesy and rancid flavors, were almost completely inhibited in the salted cream. They also found that butter produced from salted cream stored for eight days at 60 to 77° F. was superior to butter made from unsalted cream stored at 50° F. for eight days.

The data which have been published appear entirely favorable to the use of salt for improving the keeping quality of cream. Further studies using more samples of cream and a wide range of storage temperatures seemed desirable since lower storage temperatures than those which generally prevail throughout the Middlewest were used in previous studies.

## METHODS

Four series of experiments were conducted to determine the possible relationships between salt concentration, time and temperature of storage, and keeping quality of cream. In each series, which consisted of 20 samples, salt was added to 30 per cent cream in amounts equivalent to 0, 7, 10, 13 and 16 per cent of the weight of the fat-free serum and samples of the cream were held at 60, 70, 82 and 90° F., respectively, for 10-day periods. Changes in grade, acidity, and formol titration were followed, observations being made at 1, 2, 3, 4, 5, 6, 8, and 10 day periods. Changes in the bacterial flora of the 40 samples of cream in trials III and IV were followed by direct microscopic observations at the same intervals.

Samples for grading were labeled to conceal their identity, thereby eliminating much of the inevitable personal factor when organoleptic methods are employed. After each sample had been graded independently by two persons, the indicated grade was determined by mutual agreement. The grade designations used were: sweet, first, second, and third grades. The definitions for cream grades as given in the Kansas dairy law were used as a basis for grading (4). In the grading of salted cream samples, saltiness was not considered as an undesirable flavor defect.

Four trials consisting of 24 samples were conducted to determine the effectiveness of salt in preventing deterioration in cream held at 70° F. for various intervals before the salt was added. In each trial, a lot of fresh 30 per cent cream was divided into six one-pint samples. One sample was held without salt throughout the storage period, salt in 13 per cent concentration (serum basis) was added to a second sample immediately, while the remaining samples were held for 3, 4, 5, and 6 days respectively before salt in the same concentration was added. Changes in grade, acidity, and formol titrations were observed throughout the remainder of a seven-day storage period.

Three preliminary trials were conducted to determine the effect of salt in cream on the quality of the resulting butter. In each of these trials, salted cream (13 per cent salt serum basis) and control lots of the same cream without salt were held in sterile glass containers for 10 days at 70° F. The samples of cream were then neutralized if necessary, pasteurized at 150° F. for 30 minutes and churned, unsalted butter being made. The resulting butter was scored fresh and again after 60 days' storage at 0 to -10° F.

When cream to which salt had been added was tested for butterfat by the usual Babcock test procedure, two principal difficulties were encountered: (1) The reaction between the sulphuric acid and salted cream frequently caused part of the sample to boil out over the top of the test bottle, and (2) a grayish brown deposit of foreign material, which interfered with the reading of the tests, frequently occurred at the base of the fat column.

To overcome these difficulties the conventional Babcock test procedure was modified in the following respects: (1) An equal volume of cold water was added to the cream sample in the test bottle before the acid was added, and (2) the acid (17.5 ml.) was added in three equal portions with a delay of 10 minutes between the first and second additions of the acid and with a delay of five minutes between the second and third additions of the acid.

Using this modified Babcock test procedure 50 individual tests were performed on samples of salted cream by each of two operators, and the results compared with the calculated test of the samples. The calculated value was arrived at by running not less than 12 replicate tests on the unsalted cream and then calculating the theoretical test of the sample after salt was added.

Quantitative and qualitative changes in the microflora of cream were followed by direct microscopic observations (6). The method ordinarily employed for milk was used except that dilutions of one part of cream in nine of water were prepared from those samples in which the count had become so high as to make enumeration of microorganisms difficult. Observations were made only on samples of cream in series III and IV.

## RESULTS

*Effectiveness of salt added to cream immediately after separation in preventing deterioration of sweet cream held at a series of different storage temperatures.* The effectiveness of salt in retarding deterioration in sweet cream was found to be directly related to the concentration of salt used and the time and temperature of storage. The data are presented in table 1.

TABLE 1

*Change in grade of cream as affected by salt concentration and storage temperature*

Sample No.	Per cent salt (serum basis)	Grade of cream after 5 days storage (sweet, 1, 2, 3)				Grade of cream after 10 days storage (sweet, 1, 2, 3)			
		Lot I	Lot II	Lot III	Lot IV	Lot I	Lot II	Lot III	Lot IV
Cream held at 60° F.									
1a	0	2-	2-	1	1-	3	3	1-	1-
2a	7	sw	sw	sw	sw	1	1	1	1
3a	10	sw	sw	sw	sw	sw-	sw	sw	sw
4a	13	sw	sw	sw	sw	sw	sw	sw	sw
5a	16	sw	sw	sw	sw	sw	sw	sw	sw
Cream held at 70° F.									
1b	0	2-	2-	1-	1-	3	3	2	3
2b	7	1	1	1	1-	1	1	1	2
3b	10	sw	sw	sw	sw	sw-	sw	sw	1
4b	13	sw	sw	sw	sw	sw	sw	sw	1
5b	16	sw	sw	sw	sw	sw	sw	sw	sw
Cream held at 82° F.									
1c	0	3	3	2-	3	3	3	3	3
2c	7	1	1-	1-	2	2-	2-	1-	2
3c	10	sw-	1	sw	1	1	2-	2	2
4c	13	sw	1	sw	sw	1	1	1-	1-
5c	16	sw	sw	sw	sw	1-	1	sw	sw
Cream held at 90° F.									
1d	0	3	3	3	3	3	3	3	3
2d	7	1-	2	2	2	2-	2-	2	2-
3d	10	1-	1	1	1-	2-	2-	2	2
4d	13	1	sw-	sw	sw-	2	2-	2	1-
5d	16	sw	sw	sw	sw	1-	1	1	2

Control lots of cream to which no salt had been added showed rapid deterioration, the rate and extent of deterioration (measured by organoleptic grading) being most rapid at the higher storage temperatures. This is indicated by the data presented in table 1 which shows that all of the control samples became unlawful within five days at 82 and 90° F. with the exception of one sample, whereas when samples of the same cream were held at 60 and 70° F., none of the cream was unlawful and four of the eight samples were still first grade at the end of a similar storage period. After 10 days' storage all of the control samples held at 70, 82

and 90° F. were unlawful with but one exception, whereas only two of the four samples held at 60° F. were unlawful, the two remaining samples being poor first grade cream.

The addition of seven per cent salt (serum basis) to the cream held at 60° F. kept each of the four samples of cream sweet without exception for five days and from going below first grade during a 10 day storage period. When the storage temperature was increased to 70° F., all four samples containing seven per cent salt were first grade after five days and with but one exception after a 10-day storage period. This concentration of salt, however, did not prevent some of the cream from becoming second grade during a five day storage period at 82 or 90° F. One of the four samples stored at 82° F. and three of the four samples stored at 90° F. were second grade after a five day storage period. After 10 days' storage at these temperatures all of the samples with but one exception were second grade or lower.

Salt in 10 per cent concentration kept all of the cream sweet at 60 and 70° F. for a five-day period and for 10 days at 60° F. This concentration of salt prevented any of the samples from becoming second grade when held for five days at 82 and 90° F., or for 10 days at 70° F. When stored at 82 and 90° F. for 10 days only, one of the eight samples containing 10 per cent salt was first grade, the remaining samples being second grade.

Cream containing 13 per cent salt remained sweet for 10 days at 60 and 70° F. with the exception of one sample which was still first grade cream and for five days at 82 and 90° F. with the exception of one sample each, neither of which had dropped below first grade. After 10 days' storage at 82° F. two of the four samples were graded as first grade cream and the remaining two samples as poor first grade cream. After 10 days' storage at 90° F. only one of the four samples was above second grade.

All samples of cream containing 16 per cent salt (serum basis) remained sweet during five days' storage at each temperature used and during 10 days' storage at 60 and 70° F. This salt concentration was effective in keeping all samples stored at 82 and 90° F. from becoming second grade with the exception of one sample during the 10-day storage period.

*Acidity.* The results of the acidity test tend to corroborate the organoleptic grading. The rate and extent of acid development which occurred during the 10-day storage period was found to be dependent upon the salt concentration, the initial quality of the cream, and the time and temperature of storage (figure 1).

The rate of acid development was rapid during the first few days of storage in all of the control lots of cream to which no salt had been added, and was greatly accelerated at the higher storage temperatures.

Salt in 7 per cent concentration prevented any marked increase in

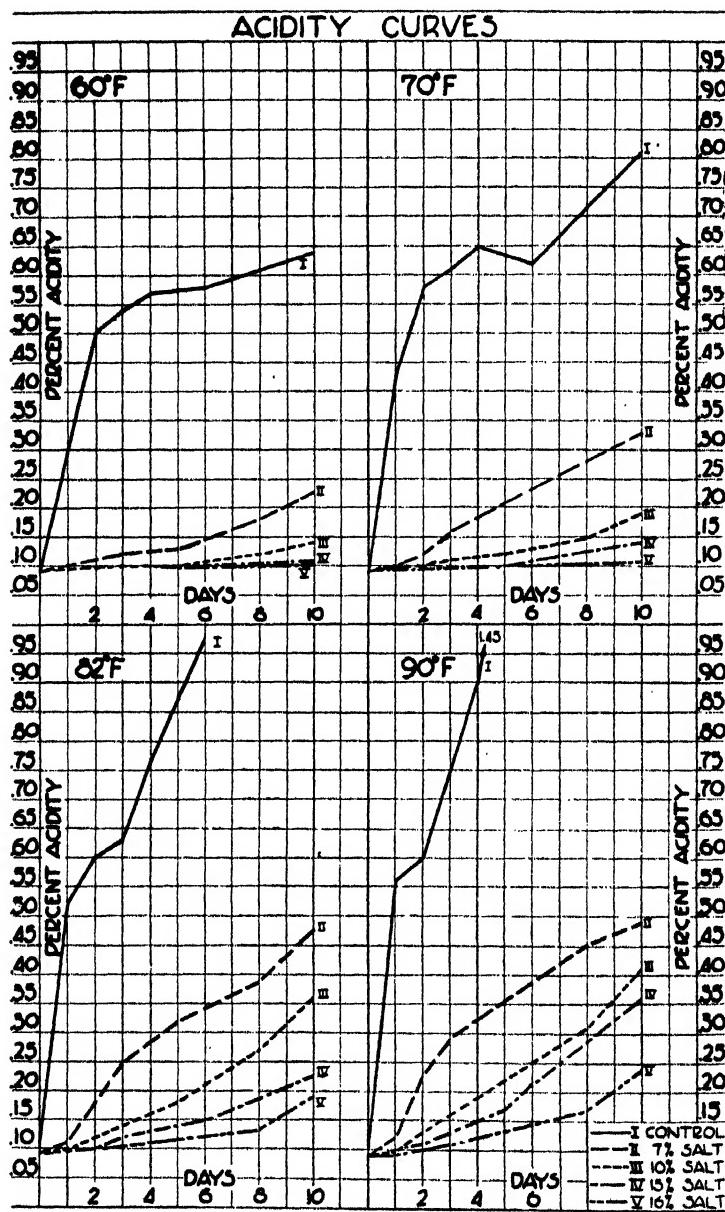


FIG. 1. Effect of salt concentration and temperature of storage on the titratable acidity of cream.

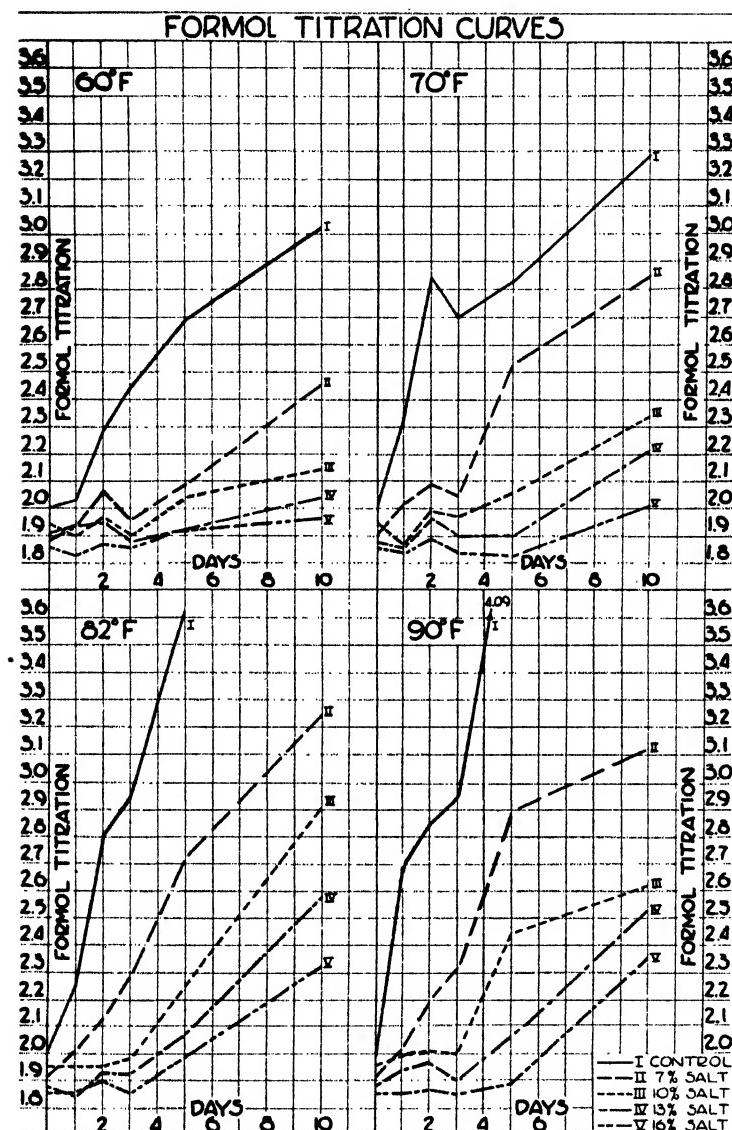


FIG. 2. Effect of salt concentration and temperature of storage on the formol titration of cream.

acidity at 60° F. throughout the 10-day storage period and greatly retarded acid development in samples stored at higher temperatures.

When 10 per cent salt was used no appreciable increase in acidity was observed after five days' storage at 60 to 70° F. and after 10 days the average acidity did not exceed 0.20 per cent at these temperatures. At 82 and 90° F., however, 10 per cent salt did not prevent the cream from becoming sour after 10 days.

Thirteen per cent salt prevented all of the samples stored at 60 or 70° F. from exceeding 0.15 per cent acid during a 10-day period and was effective for five days at 82 and 90° F. in preventing the acidity from increasing above 0.17 per cent. After 10 days the average acidity was 0.23 per cent for the samples stored at 82° F. and 0.36 per cent for the samples stored at 90° F.

When 16 per cent salt was used no appreciable increase in acidity occurred in the samples stored at 60 or 70° F. for 10 days. Although there was some increase in acidity at 82 and 90° F., the maximum acidity observed was 0.24 per cent, indicating the effectiveness of this salt concentration in retarding acid development at all of the storage temperatures.

*Formol titration.* The effectiveness of salt in preventing proteolytic decomposition of the cream as measured by the formol titrations (figure 2) reflect the same general trends that were shown by changes in acidity (figure 1). The data show that proteolytic decomposition was more rapid in the control samples and that the rate and amount of decomposition was accelerated by an increase in the storage temperatures.

Addition of seven per cent salt did not prevent an appreciable increase in the formol titration values during a 10-day holding period at any of the temperatures used. Ten per cent or more of salt was effective at 60 and 70° F. in preventing any marked increase in the formol titration values during 10 days, but was considerably less effective at 82 and 90° F. Thirteen per cent salt prevented any marked change in the formol titration during five days at all temperatures used, but did not prevent a considerable increase after 10 days at 82 and 90° F. When 16 per cent salt was used no increase in the formol titration value of the cream was observed during five days at all temperatures or after 10 days at 60 and 70° F. However, the formol titration values increased from 1.86 to 2.33 at 82° F. and from 1.86 to 2.36 at 90° F. during the storage period. Data indicate that none of the concentrations of salt used were entirely effective in preventing increases in the formol titration values at the higher storage temperatures.

*Bacteriological.* The changes in the numbers of bacteria in the various lots of cream in series III and IV are shown in figures 3 and 4. In all cases the counts of the control samples reached levels of a billion or more, the time required to reach this level being longer at the lower temperatures.

At 82 and 90° F. the flora changed from cocci to rods as the holding period increased toward the time when the cream became second or third grade. In some instances appreciable numbers of yeast were found as would be expected from the development of yeasty flavors in some samples.

The addition of salt markedly retarded the development of bacteria, the greater the amount of salt the greater being the inhibitory action. In the presence of the salt the numbers of bacteria were never found to reach the large numbers which were characteristic of the maxima of the control

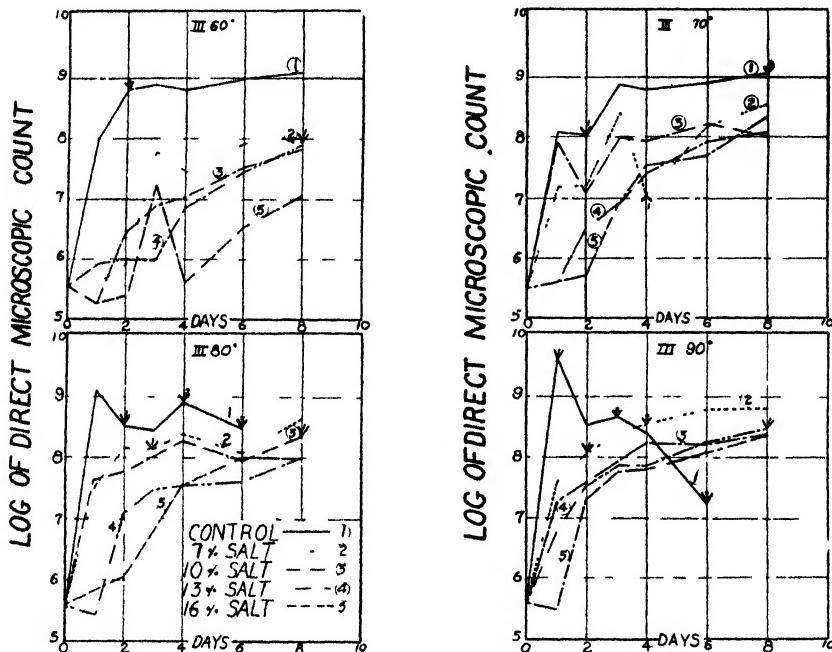


FIG. 3. Effect of salt concentration and temperature of storage on the direct microscopic count of cream samples of Lot III. (1 indicates change of grade.)

samples. In the samples containing salt the flora was largely micrococci, especially in those containing the larger amounts of salt. A few short-chain streptococci, possibly enterococci, were found in the samples containing 7 and 10 per cent salt. Not only does the added salt inhibit the growth of bacteria; it also influences the types of organisms which develop. Both of these factors undoubtedly are responsible for the markedly improved keeping quality of cream to which salt in appropriate quantity has been added.

*Effectiveness of salt in stopping deterioration of cream.* The addition of 13 per cent salt on the serum basis to cream held at 70° F. for three or more days before the salt was added did not prevent further deterioration

of the cream as measured by organoleptic grading of the samples (table 2). The samples to which the salt was added after three or more days of storage generally graded as low or lower than the corresponding control lots of cream to which no salt had been added. On the other hand, the cream

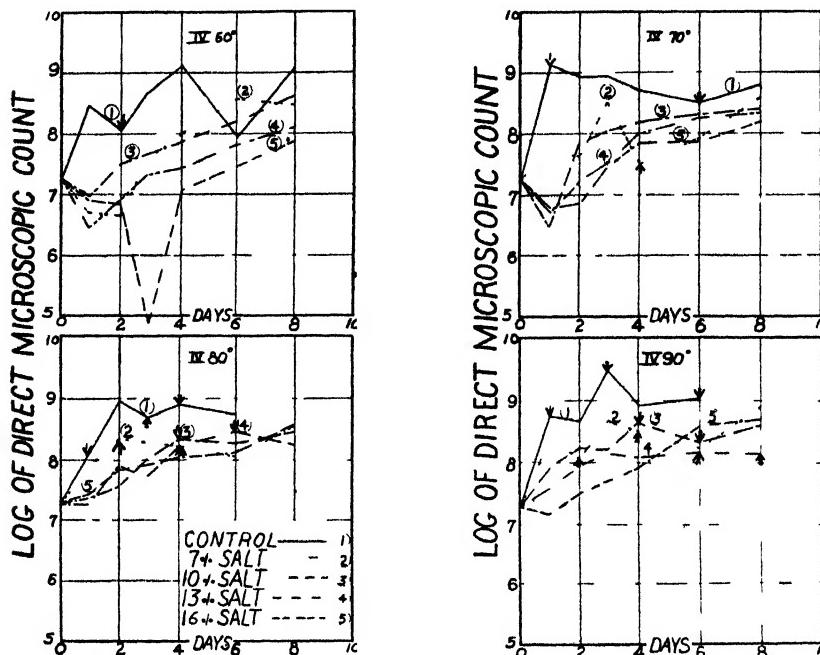


FIG. 4. Effect of salt concentration and temperature of storage on the direct microscopic count of cream samples of Lot IV. (\* indicates change of grade.)

TABLE 2

*Change in grade of cream as affected by adding salt at varying intervals of time during storage at 70° F.*

Time when salt was added	Per cent salt added (serum basis)	Grade of cream after 7 days storage			
		Lot I	Lot II	Lot III	Lot IV
Check (no salt)	0.0	2	1-	2	2-
Immediately	13.0	sw	sw	sw	sw
After 3 days	13.0	1-	1-	2	3
After 4 days	13.0	1-	2	2	2-
After 5 days	13.0	2	2	2-	3
After 6 days	13.0	2-	1-	2	3

to which the salt was added immediately after separation remained sweet in each of the trials. The data indicate that the salt must be added to the cream soon after separation to be effective in preventing deterioration.

*Effect of salt preservation of cream on quality of butter.* Butter churned from cream to which 13 per cent salt on a serum basis had been added before holding for 10 days at 70° F. scored from two to five points higher than did the butter produced from control lots of the same cream held without salt (table 3). The butter churned from the salted creams scored 92, 92,

TABLE 3  
*Effect of salt in cream on the score of the resulting butter  
(Cream stored at 70° F. for 10 days)*

Lot number	Butter score—freshly churned			Butter score—after storage*		
	Salted cream†	Unsalted cream	Difference	Salted cream†	Unsalted cream	Difference
1	92.0	87	5.0	92.0	87	5.0
2	92.0	90	2.0	92.0	90	2.0
3	92.5	90	2.5	92.5	90	2.5
Average	92.2	89	3.2	92.2	89	3.2

\* Butter stored for 60 days at 0° F. to -10° F.

† Thirteen per cent salt added, serum basis.

and 92.5, respectively, whereas the control samples scored 87, 90, and 90, respectively. There was no change in score of any of the samples after a 60-day storage period of 0 to -10° F., indicating that there was no appreciable tendency for chemical change in the butter produced from the salted cream. The samples of butter were analyzed for salt and in no instance was the salt content in excess of 2.5 per cent.

Although the data presented represent only three lots of cream, they do indicate that considerable improvement in the quality of butter churned from cream held without adequate cooling for considerable periods of time can be obtained by the addition of a suitable quantity of salt to the cream.

*Effect of salt in cream on the accuracy of the Babcock test.* The results of the modified Babcock test on salted cream are shown in table 4. Nearly half of the tests (45 per cent) checked within 0.2 per cent of the calculated test, and approximately 80 per cent of the tests checked within 0.5 per cent of the calculated test. In only two of the 100 tests did the results vary as much as 1.0 per cent or more from the calculated test. The variations between 50 individual tests of salted cream by each of two operators and the calculated fat percentages were no greater for one operator than for the other. Seventy-six per cent of the tests by operator No. 1 and 82 per cent of the tests by operator No. 2 were within 0.5 point of the calculated test.

The data indicate that salted cream samples may be tested with about the same degree of accuracy as is now attained with the conventional Babcock test procedure for unsalted cream. The modified test proposed

TABLE 4

*Variations between the actual and calculated fat percentages of salted cream*

Variation from the calculated test in points	Operator 1	Operator 2	Total No. of tests	Per cent of tests	Cumulative per cent of tests
	No. of tests	No. of tests			
0.0	2	8	10	10	10
0.1	17	10	27	27	37
0.2	6	2	8	8	45
0.3	4	14	18	18	63
0.4	7	1	8	8	71
0.5	2	6	8	8	79
0.6	2	4	6	6	85
0.7	8	0	8	8	93
0.8	1	4	5	5	98
0.9	0	0	0	0	98
1.0	0	1	1	.1	99
1.1	1	0	1	1	100
Total	50	50	100	100	..

Note: A modified Babcock test procedure was used in testing the salted cream samples.

for salted cream does have the disadvantage of being more time-consuming than the conventional Babcock test for unsalted cream.

#### CONCLUSIONS

1. The results obtained in this study indicate that deterioration in cream held in glass containers as measured by organoleptic grading, acid development, formol titration, and direct microscopic examination may be definitely retarded by the addition of salt immediately after separation.

2. The amount of salt necessary to effectively prevent deterioration in cream was found to be dependent upon the time and temperature of storage. The results indicate that not less than 10 per cent salt (serum basis) would be required to prevent cream from becoming second grade when held for 10 days at 60 or 70° F. or for five days at 82 and 90° F. Since most cream is delivered within five days after it is produced, the addition of not less than 10 per cent salt (serum basis) should be adequate to enable the producer to market either sweet or first grade cream.

3. The addition of 13 per cent salt (serum basis) to cream held at 70° F. for three or more days before the salt was added did not prevent further deterioration of the cream. Thus the method is limited largely to farm use.

The above results were obtained by holding the cream in glass containers. In their interpretation consideration should, therefore, be given to the possible effects of prolonged exposure in the metallic cream container, to salted cream and to the corrosive action of the brine on the can, which may shorten the life of the can and in addition may cause metallic action damaging to the flavor and keeping quality of the cream and of the resulting butter.

4. Butter churned from cream to which 13 per cent salt (serum basis) was added at the beginning of a 10-day storage period at 70° F. scored two to five points higher than did butter produced from control lots of the same cream held under similar conditions without salt. In no case did the salt content of the butter churned from cream to which 13 per cent salt had been added exceed 2.5 per cent.

5. When a modified Babcock test procedure was used, results which agreed favorably with the calculated butterfat percentages were obtained.

6. Data obtained in this study as well as studies by other investigators indicate that the preservation of cream by the addition of salt has definite merit. Since salt is used in the preservation of many other foods and is a normal constituent of butter as usually marketed, the condemning of cream which contains added salt does not seem to be justified.

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# THE SPECIFICITY OF THE LACTOGENIC HORMONE IN THE INITIATION OF LACTATION<sup>1</sup>

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In the recent literature Folley and Young (1, 2) have raised two questions in regard to the relation of the physiological activity of the "lactogenic" factor (as assayed with pigeons) and other hormones of the anterior pituitary in stimulating the lactation process. The first question was whether the factor stimulating the crop gland of the pigeon was identical with the factor which stimulates lactation in mammals and the second question was whether there were not several factors in the anterior pituitary which possessed lactogenic properties. It was suggested that the glycoprotic factor has this property. They concluded that "*those extracts which stimulate the secretion of normal milk in the mammal should be described as containing a lactogenic substance or substances.*"

The writers (3) recently presented data indicating that lactogenic preparations as assayed with pigeons and with rabbits gave comparable potency, and evidence was presented which indicated that lactogenesis and pigeon crop gland proliferation were due to the same factor. A similar conclusion was reached by Lyons (4) in comparison of pigeon crop gland proliferation and lactation stimulation in estrus guinea pigs.

The second question raised represents a difference of opinion as to the definition of a "lactogenic" hormone. We believe that the term "lactogenic" should be reserved for hormones which will initiate lactation in non-lactating mammary glands of animals when conditioned for lactation (by previous pseudo-pregnancy, estrus, estrogen and progestin or the mammogens). In other words, the hormone should stimulate lactogenesis rather than augment established lactation.

The object of the present paper is to present data indicating that if the term "lactogenic" hormone is restricted to anterior pituitary extracts which will initiate lactation in animals with glands conditioned to lactate, then there is only one lactogenic hormone and that fraction proliferates the pigeon crop gland.

## EXPERIMENTAL

In a previous paper (5) a method of separating an initial anterior pituitary extract into two fractions, one rich in the lactogenic hormone and the

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other containing the thyrotropic and other hormones was described. The extracts used were prepared from cattle pituitaries.

The "lactogenic hormone" fraction was assayed by the McShan-Turner (6) method and contained 5000 pigeon units per gram. Only traces of the other pituitary hormones were present (7). The "thyrotropic and other hormone" fraction was rich in the thyrotropic hormone and contained 4000 Bergman-Turner (8) guinea pig units per gram. It also contained 638 Bergman-Turner (9) guinea pig units of carbohydrate metabolism hormone, 2500 Bergman-Houchin-Turner (7) chick units of gonadotropic hormone, 100 McShan-Turner (6) pigeon units of lactogenic hormone and 16.7 Houchin-Turner (10) guinea pig units of fat metabolism hormone per gram of extract.

It seemed of interest to determine the ability of these two extracts to initiate lactation in pseudo-pregnant rabbits. If the thyrotropic fraction would also initiate lactation in such animals, yet was practically free of pigeon crop gland stimulating potency, it would furnish good evidence of the individuality of lactogenesis in contrast to crop gland effect and the presence of at least two lactogenic hormones.

The extracts were all injected at their iso-electric point. The dried powder was dissolved in a slightly alkaline solution with NaOH, then precipitated by bringing the solutions to their iso-electric point with HCl. The concentration of the fluid was 1 per cent (1 cc. = 10 mg.).

*Lactogenesis with "Lactogenic Hormone" Fraction.* The lactogenic hormone fraction was assayed for its ability to initiate lactation in the pseudo-pregnant rabbit according to the Gardner-Turner (11) method. When injected at the rate of 1 mg. per 100 gms body weight the glands were mostly (+) to (++)<sup>1</sup>, the average rating being 1.67; when 1.5 mg. was used (+++) to (++++) glands were obtained, the average rating being 3.17, which is considered the unit response (3). At this level approximately 52 mg. containing 260 McShan-Turner pigeon units in rabbits weighing an average of 3400 gms. was required. It thus appeared that about 1.5 mg. of the lactogenic hormone per 100 gm. body weight would initiate copious lactation in rabbits.

*Lactogenesis with the "Thyrotropic and Other Hormones" Fraction.* With the potency of the "lactogenic hormone" established for lactogenesis in rabbits, the next step was to determine whether equivalent dosages of the thyrotropic fraction would initiate lactation in pseudo-pregnant rabbits. Five rabbits were injected at the rate of 2 mg. per 100 gms. body weight. One animal died the second day. The other 4 animals showed no signs of lactation, not even duct lactation, which occurs in a low percentage of pseudo-pregnant rabbits. From these results it is clear that lactation was not initiated by amounts of the thyrotropic extract which were sufficient, in the case of lactogenic fraction, to stimulate maximum distension of the glands with milk (table 1).

Eleven rabbits were then injected with the thyrotropic fraction at the rate of 5 mg. per 100 gms. body weight. Several of these animals died after a single injection. Six animals survived the test period and of these four showed no signs of lactation. Only a single animal showed some evidence of stimulation (++) beyond that occasionally seen in pseudo-pregnant animals (table 1).

The cause of death of a considerable number of the animals is believed to be due to the large amount of the thyrotropic hormone which the extract contained. The animals appeared especially susceptible when they were kept in a warm room or during warm days in the spring. It is rather surprising, however, that the action would be so rapid; a number of animals died following the first injection.

The third group of rabbits was injected at the rate of 10 mg. per 100 gms. body weight. Special precautions were taken to keep the animals outside and at a relatively low temperature. Of six animals injected at this level, only one showed any signs of lactation. An explanation for the rather good lactation observed in this one case cannot be advanced. It is

TABLE 1  
*Lactation response in normal pseudo-pregnant rabbits with the "thyrotropic and other hormones" fraction*

Rabbit No.	Body weight	Amt. injected 100 gm. body weight	Total amount injected	Bergman-Turner thyrotropic units (guinea pigs)	McShan-Turner lactogenic units	Gardner-Turner rating of lactation
	gms.	mg.	mg.			
1	3050	2	62	248	6.2	0
2	2800	2	...	...	...	Died 2nd day
3	4000	2	80	320	8.0	0
4	3840	2	76	304	7.6	0
5	2760	2	56	224	5.6	0
1	3550	5	180	720	18	+ Died 6th day
2	3430	5	170	680	17	++
3	3920	5	...	...	...	Died 1st day
4	2790	5	140	560	14	0
5	3650	5	185	740	18.5	0 to +
6	3680	5	...	...	...	Died 1st day
7	3430	5	...	...	...	Died 1st day
8	3830	5	190	760	19.0	0
9	3740	5	185	740	18.5	0
10	3970	5	200	800	20.0	0
11	3210	5	...	...	...	Died 2nd day
1	3080	10	300	1200	30	+++
2	3560	10	360	1440	36	0
3	3470	10	350	1400	35	0 to +
4	3280	10	330	1320	33	0
5	3810	10	380	1520	38	0
6	3410	10	340	1360	34	0 to +
1	2950	20	400	1600	40	++ Died 4th day

possible that a few rabbits are responsive to much less lactogenic hormone than is usually required.

Finally, a single rabbit was injected with 20 mg. per 100 gms. body weight. Although this animal died on the 4th day of injection, it showed signs of lactation.

From this series of experiments, it seemed reasonable to draw the conclusion that the "thyrotropic and other hormones" fraction of the anterior pituitary does not contain the factor for lactogenesis when injected in amounts five to ten times as great in weight as the requirement of the "lactogenic" fraction to induce maximum lactation. In the maximum dosages, the units of the "lactogenic" hormone present as a contaminant was low and insufficient to stimulate lactation. At the higher levels injected, many of the rabbits were unable to tolerate the thyrotropic hormone except during periods of cold weather when the excess heat produced could be dissipated.

It might be said, however, that the lactogenesis of the thyrotropic fraction was being masked by the excess of the thyrotropic hormone. The excess metabolism induced would be unfavorable to the lactation process. One might say that if the thyrotropic hormone were removed, the other factors would be able to show their influence. As we did not have a preparation free of the thyrotropic hormone, yet containing the other factors, it was decided to thyroidectomize the rabbits and thus free them of the metabolic upset which follows large injections of this fraction.

*Lactogenesis in Thyroidectomized Rabbits* Five rabbits were thyroidectomized, three days before the end of pseudo-pregnancy. The injection of 5 mg. of the "thyrotropic and other hormones" extract per 100 gms.

TABLE 2

*Lactation response in thyroidectomized pseudo pregnant rabbits with the "thyrotropic and other hormones" fraction*

Rabbit No.	Body weight	Amt. injected 100 gm. body weight	Total amount injected	Bergman Turner thyro tropic units (Guinea pigs)	McShan Turner lactogenic units	Gardner Turner rating of lactation
	gms.	mg.	mg.			
1	2825	5	140	560	14	++
2	3020	5	150	600	15	0
3	3710	5	185	740	18.5	0
4	4100	5	205	820	20.5	0 to +
5	3360	5	170	680	17	++
1	2680	10	270	1080	27	+
2	2800*	10	280	1120	28	++
3	3600	10	360	1440	36	++
4	3170	10	320	1280	32	++
5	3500	10	350	1400	35	+
6	3340	10	330	1320	33	+
7	3030	10	300	1200	33	+

body weight initiated (++) lactation in two animals but little or none in the other three (table 2).

Seven other similar rabbits were injected at the rate of 10 mg. per 100 gms. body weight. These animals all showed at least duct lactation (+) with three showing further progress (++) in the filling of the gland with milk. As the units of the "lactogenic" hormone present as a contaminant in the extract were still far less than that required to induce a similar stage of lactation in normal animals, it would appear that other factors in the pituitary were able to supplement and intensify the apparent activity of the lactogenic hormone present.

A tentative theory to explain this phenomenon follows: On the assumption that a true lactogenic hormone is required to condition the epithelial cells of the mammary gland to synthesize milk, it indicates that without the hormone no lactation would result. On the other hand, it might require less lactogenic hormone to develop a certain stage of filling of the gland, if, once the cells were activated, other hormonal factors stimulated the rate of milk secretion by the epithelial cells.

As the "thyrotropic and other hormones" fraction contained considerable amounts of the factor which elevates the blood sugar, it seems reasonable to believe that this hormone would augment lactation once established and at the end of the test period of six days show a degree of lactation greater than would be obtained by an equivalent amount of pure lactogenic hormone. As a matter of fact it seems possible that the pituitary contains several lactation augmenting hormones. Even the thyrotropic hormone in suitable dosage would be expected to augment lactation considering the demonstrated influence of thyroxine on lactation (12, 13).

*Supplementing Effect of Pituitary Hormones on Lactation.* If one assumes that the initiation of lactation by the epithelial cells of the mammary gland is stimulated by the lactogenic hormone whereas other pituitary hormones augment the rate of the lactation process thus established, it should be possible to obtain higher lactation ratings in the pseudo-pregnant rabbits with a minimum of lactogenic hormone if supplemented with the other fraction. It was decided, therefore, to determine the effect of injecting one mg. of the lactogenic hormone, which when injected alone produced an average lactation rating of 1.67, with one mg. of the "thyrotropic and other hormones." The seven rabbits which survived showed a slight augmentation of lactation, averaging 1.86 (on the Gardner-Turner rating).

In a second group of five rabbits, the "thyrotropic" fraction was increased to two mg. per 100 gms. body weight, the lactogenic remaining the same as previously (1 mg.). These animals showed (++) to (+++) ratings, averaging 2.60. In other words, with lactogenic hormone alone about 260 McShan-Turner pigeon units were required to induce (++) lactation, whereas in these experiments about 100 units less of the lactogenic hormone

were required to produce the same degree of gland filling when the other hormones of the pituitary supplemented its action.

#### DISCUSSION

Azimov and Krouze (14) were the first to point out the apparently paradoxical fact that crude bovine pituitary preparations were able to increase established lactation in dairy cattle to a greater extent than the more highly purified lactogenic hormone fraction. Folley and Young (1) confirmed this observation and further obtained a substantial temporary increase in milk volume with cows injected with a "thyrotropic" preparation which contained no detectable amount of the substance that stimulates growth of the pigeon crop gland. From these and other observations Folley (15) has questioned the specificity of the lactogenic hormone when assayed by the pigeon crop gland. It is his opinion that extracts which stimulate the secretion of normal milk in mammals should be described as containing the lactogenic substance.

The writers (3) have recently presented evidence which they interpret as indicating that the extract of the pituitary which is commonly called the "lactogenic hormone" and which possesses the ability to proliferate the pigeon crop gland also possesses the ability to *initiate* lactation in pseudo-pregnant rabbits to a high degree. In the present paper it was shown that the extract remaining, rich in the thyrotropic and other hormones but containing little "lactogenic" hormone would only rarely *initiate* lactation in dosages as high as could be tolerated.

It is the opinion of the writers that the confusion which the papers of Folley and Young may have created in the minds of some may be eliminated by a suitable definition of the term *lactogenic*. We suggest that the term *lactogenic* be reserved for the hormone or hormones which have the ability to initiate secretory activity in the epithelial cells of the mammary gland as contrasted with the terms *galactogogue* or *galactopoetic* for substances which have the ability to augment the rate of established lactation. By these definitions, the hormones of the anterior pituitary and of other endocrine glands which directly and indirectly influence the lactation process may be divided into two groups. The lactogenic hormone is believed to act directly upon the epithelial cells of the mammary gland initiating and maintaining the secretory activity of the cells in the presence of suitable amounts of the milk precursors. This hormone will initiate lactation in the glands of rabbits, guinea pigs and other animals when they are conditioned by the mammogenic hormones. It also stimulates the proliferation of the pigeon crop gland. The experiments with hypophysectomized animals (16) indicate the need of the hormone continuously during lactation. It is true that the lactogenic hormone may also augment established lactation when the rate of secretion of the hormone is less than optimal in relation to the precursors

TABLE 3

*Lactation response in normal pseudo-pregnant rabbits with supplementing effect of pituitary hormones*

Rabbit No.	Body weight	Amt. injected 100 gm. body weight	Total amount injected	Bergman-Turner thyrotropic units (guinea pigs)	McShan-Turner lactogenic units	Gardner-Turner rating of lactation
	gms.	mg.	mg.			
1	3260	1 Th*	33	132	3.3	
		1 Lac†	33		165.0	++
2	3430	1 Th	34	136	3.4	
		1 Lac	34		170.0	+
3	3220	1 Th	32	128	3.2	
		1 Lac	32		160.0	+
4	2820	1 Th	29	116	2.9	
		1 Lac	29		145.0	+++
5	3460	1 Th				
		1 Lac				Died 2nd day
6	2700	1 Th	27	108	2.7	
		1 Lac	27		135.0	+++
7	4230	1 Tt	42	168	4.2	
		1 Lac	42		210.0	+
8	2780	1 Th	28	112	2.8	
		1 Lac	28		140.0	++
Ave. of seven	3206	1 Th	32.1	128.4	3.2	1.86
		1 Lac	32.1		160.5	
1	3910	2 Th				
		1 Lac				Died 1st day
2	2970	2 Th	60	240	6.0	
		1 Lac	30		150.0	+++
3	3520	2 Th	70	280	7.0	
		1 Lac	35		175.0	+++
4	3190	2 Th	64	256	6.4	
		1 Lac	32		160.0	+++
5	4270	2 Th	86	334	8.6	
		1 Lac	43		215.0	++
6	2690	2 Th	54	216	5.4	
		1 Lac	27		135.0	++
7	3910	2 Th				Died 5th day
		1 Lac				++ rating
Ave. of five	3328	2 Th	66.8	267.2	6.7	2.60
		1 Lac	33.4		167.0	

\* Th = "thyrotropic and other hormone" fraction.

† Lac = "lactogenic hormone" fraction.

of milk. Consequently, in established lactation it is impossible to distinguish the lactogenic from galactogogic hormones.

The mode of action of the galactogogic hormones is believed to be indirect through their ability to mobilize larger quantities of the precursors of milk and to increase the metabolism of the cells. These hormones affect many bodily processes and their ability to augment the rate of milk secretion is solely due to the ability of the mammary gland to take advantage of

the improved general metabolism of the body. In other words, in the absence of the lactogenic hormone no lactation would occur even with increased mobilization of milk precursors.

The writers have reason to believe that the anterior pituitary secretes a number of hormones which individually and collectively have the ability to augment the lactation rate. Further, it has been suggested that the differences in the productive ability of dairy cattle is due in part to the differences in the rate of secretion of these hormones and the glands and hormones which they influence (17). The outstanding example of this relationship is the thyrotropic hormone which stimulates the secretion of thyroxine. Thyroxine in turn has been shown to have a marked influence in augmenting the rate of established lactation (13). The pituitary hormones also influence the adrenal gland and the metabolism of fat, carbohydrate, protein and mineral matter. As these factors are separated from the mixture of hormones, the augmentation of lactation due to their joint action may be separately evaluated.

#### SUMMARY

A study is reported with the "lactogenic hormone" and the "thyrotropic and other hormone" fraction of the anterior pituitary on lactogenesis in the pseudo-pregnant rabbit.

When the "lactogenic hormone" fraction was injected at the rate of 1 mg. per 100 grams body weight (+) to (++) glands were obtained, the average rating being 1.67. When 1.5 mg. was given (+++) to (++++) glands were obtained the average rating being 3.17, which is considered in the range for a unit response. At this level approximately 52 mg. containing 260 McShan-Turner pigeon units were required.

A group of rabbits injected with 2 mg. per 100 gm. body weight of the "thyrotropic and other hormone" fraction showed no evidence of lactation. At the 5 mg. and 10 mg. levels six animals in each group survived the test period. Only one rabbit in each group showed any evidence of lactation.

In a group of five thyroidectomized rabbits injected with 5 mg. of the "thyrotropic and other hormone" fraction per 100 grams body weight (++) lactation was initiated in two animals but little or none in the other three. Another group of seven thyroidectomized rabbits injected at the 10 mg. level gave mostly (+) to (++) glands, the average rating being 1.43. All the animals survived the test period.

The average gland rating was increased from 1.67 for 1 mg. of lactogenic hormone per 100 grams body weight, to 1.86 when supplemented by 1 mg. of the "thyrotropic and other hormone" fraction. When the level of injection of the latter was 2 mg. the average gland rating was increased to 2.60.

These results are taken to indicate that the primary function of the lac-

togenic hormone, which also possesses the ability to proliferate the pigeon crop gland, is to initiate and maintain established lactation. Extracts rich in the thyrotropic and other hormones, but containing only traces of the lactogenic hormone, do not possess the ability to initiate lactation in doses as high as could be tolerated. This fraction, however, has a supplementing effect on established lactation.

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## ERRATA

*Vol. XXIII, No. 1, page 66, table 9.* The third column total should read 61,038.92 rather than 45,038.92.

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# JOURNAL OF DAIRY SCIENCE

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## ABSTRACTS OF LITERATURE

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The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

## ABSTRACTS OF LITERATURE

### BACTERIOLOGY

- 1. A Study of the Effect of the Growth of Some Organisms in Milk on the Phosphatase Test.** CHARLES PALEY, Certified Laboratories, Inc., New York City. *J. of Milk Techn.,* 2: 251-253. 1939.

The organisms *B. lacticus*, *S. aureus*, *E. coli* (2 strains), *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *S. lactis*, *B. subtilis*, and *S. albus*, were studied and found to have no effect upon the phosphatase test when made by either the Gilcreas-Davis modification of the Kay and Graham method or by the Scherer method.

L.H.B.

- 2. The Sterilizing Quality of Chlorine Solutions Under Different Conditions.** F. M. SCALES AND MURIEL KEMP, Sheffield Farms Research Laboratory, New York City. *J. of Milk Techn.,* 2: 215-221. 1939.

The pH and temperature of a chlorine solution are important factors affecting its germicidal efficiency. A pH around 6.0 proved better than one around 10.0 or 11.0. In laboratory and plant tests a solution containing about 50 p.p.m. of available chlorine at a pH around 6.0 proved to be as satisfactory as one containing 255 p.p.m. of chlorine at a pH of about 10.0. Acid sodium phosphate is satisfactory for use in adjusting the pH.

A temperature of 90° F. proved more effective than did a lower temperature when the pH of the solution was 8.0 or under.

Two minutes exposure seemed too short for dependable sterilizing action when solutions containing 50 p.p.m. available chlorine were used even at acid pH values. Five minutes was generally satisfactory.

Cultures of *S. aureus* and mixed cultures were used in the tests.

L.H.B.

### CHEESE

- 3. The Salting and Cooking of Curds in the Manufacture of Several Varieties of Cheeses.** J. C. MARQUARDT, N. Y. State Agr. Exp. Sta., Geneva, N. Y. *Techn. Bull. No. 670.* July, 1936.

A study has been completed associating the composition and quality of five varieties of cheese with variations in salting and cooking methods.

The study revealed that cheese curds should be salted at a rate based upon the milk fat content of the milk used.

Cook variation studies added fundamental knowledge useful for further investigations dealing with cheese improvement.

The study indicated that comparable milks made into cheese produced quality cheeses upon the basis of the cheese variety made, with Cheddar first followed in order by granular, Monterey, and brick.

The study indicated that quality and uniformity could not be regularly expected when making cheese by the Camosun method from the type of milk generally available.

J.C.M.

**4. Methods for Determining Salt in Various Cheeses.** J. C. MARQUARDT, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Techn. Bull. No. 249. Sept., 1938.

The reliability of results obtained with the modified Volhard procedures for analyzing cheeses for salt has been established for several varieties of cheeses.

A simple procedure is described for rapidly determining by direct titration the salt percentage in several cheese varieties when less than 5 days old.

Comparable results were obtained when using potassium chromate and dichlorofluorescein as indicators for the direct titration.

Failure to attain reliable results with certain well-cured cheeses by a direct titration method has been studied. The importance of soluble protein, time in solution, temperature of solution, and reaction have been reported upon.

J.C.M.

**5. Methods of Making Cheddar Cheese from Milk with a Low Curd Tension Due to Latent Mastitis.** J. C. MARQUARDT AND G. J. HUCKER, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Techn. Bull. No. 242. 1937.

It is generally agreed that a high quality cheddar cheese cannot be made from milk produced by cows with an active mastitis infection. However, since the udder tissues of practically all cattle harbor the streptococcus associated with the common type of bovine mastitis, the infection exists either in a latent or a chronic condition in almost all producing herds.

This investigation has shown that even where the milk contains demonstrable numbers of mastitis streptococci and more than 500,000 leucocytes per cc., the milk can be made into satisfactory cheddar cheese though it may lack in normal curd-formation properties. This was accomplished by the addition of 1½ to 3 per cent of starter, or by the addition of 30 per cent hydrochloric acid at the rate of 100 cc. per 1,000 pounds of milk with a smaller amount of starter.

The study has revealed the necessity and importance of using a test like the Marschall cup test in making cheese from milk whose curd tension varies from normal. It has been established that after 9 months of curing, *Streptococcus agalactiae* were present in the cheeses made from the experimental milk with a low curd tension.

Unless even latent or chronic conditions of mastitis are eliminated from cheese milk herds or the milk is especially handled during the making, losses may be experienced.

J.C.M.

- 6. Cheeses of New York State.** C. D. KELLY AND J. C. MARQUARDT, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Circular No. 174. Aug., 1937.

The varieties of cheese made in New York State are briefly described.

T.S.S.

- 7. Pasteurization of Milk for Cheese Making.** C. D. KELLY, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Circular No. 175. Aug., 1937.

Reasons for pasteurizing milk for cheese making from the public health and cheese quality aspects are discussed. The development of pasteurization in the cheese industry is briefly outlined. Some of the problems encountered in making cheese from pasteurized milk are presented. The possible value of pasteurization as a means of maintaining uniform high quality in cheese is pointed out.

T.S.S.

#### DISEASE

- 8. Mastitis. IV. The Composition of Milk as Affected by Latent Mastitis.** A. C. DAHLBERG, J. J. KUCERA, J. C. HENING AND G. J. HUCKER, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Techn. Bull. No. 239. 1936.

Earlier investigations indicate that the chemical composition of abnormal mastitis milk is quite different from that of milk normal in appearance. More recent work indicates that a latent mastitis infection may be present in the udder without causing any visible change in the milk. The present investigation was undertaken to determine whether there is a demonstrable relationship between the degree of infection and the chemical composition of milk normal in appearance.

Only cows having udders free from active inflammation and whose milk was normal in appearance were selected for study. These cows were divided into three groups, *viz.*, (A) no demonstrable infection, (B) slight infection and (C) pronounced latent infection but milk normal in appearance.

Composite milk samples of complete milkings from each of these groups were submitted to detailed chemical analysis. During the course of the study samples of fore-milk from each quarter of each cow were studied by bacteriological test to determine the amount of infection present.

Only slight differences in the chemical composition of the mixed milk of these groups were found, regardless of the degree of infection, as long as the milk remained normal in macroscopic appearance. The slight differences in chemical composition included a decrease in lactose, specific gravity, skim-milk solids and curd tension while the chlorides and albumins were slightly increased. These changes in composition were not greater than variations in chemical composition of milk between two herds of the same breed.

It is concluded that milk normal in appearance is essentially normal in

chemical composition. If no milk is excluded from inflamed, congested or injured quarters, the chemical composition of the milk from a herd will be normal in chemical composition.

It follows that earlier investigations on the chemical composition of normal milk were not affected by the possible presence of latent mastitis.

Authors' Abstract.

**9. Mastitis. VI. The Effect of Feeding Irradiated Yeast on the Resistance of the Udder to Bovine Mastitis.** G. J. HUCKER AND MARION SNYDER REED, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Techn. Bull. No. 243. 1937.

To determine the effect of irradiated yeast on the resistance of dairy cows to udder infections, 116 cows in four dairy herds were studied. Fifty-one were fed irradiated yeast in the grain mixture. In three herds 9 ounces per day per cow and in one herd 4½ ounces per day per cow were fed.

Prior to the beginning of the feeding of the yeast, weekly quarter samples from all cows were subject to laboratory examination over a 4-month period to determine the amount of mastitis infection present. Examinations were made for presence of mastitis streptococci, number of leucocytes per cc., reaction to brom thymol blue, and physical appearance of the milk. Subsequent to the initiation of the yeast feeding, similar samples were secured for approximately 20 months. In all, the observations were made on the experimental herds over a period of 2 years.

In general the feeding of irradiated yeast was found to have no significant effect upon the resistance of the udder to the invasion of mastitis streptococci. Depending upon the index of infection used, from 10 to 13 per cent more of the infected yeast-fed cows showed an improvement than was found in the case of the infected cows not fed yeast.

No prophylactic effect was found by the addition of irradiated yeast to the diet of mastitis-free cows.

Authors' Abstract.

**10. Mastitis. VII. The Relation of Bovine Mastitis to Milk Production.** G. J. HUCKER, MARION SNYDER REED AND E. S. SAVAGE, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Techn. Bull. No. 244. July, 1937.

The investigation was planned to secure definite information on the relationship between udder infections and the total amount of milk and milk fat produced. Thirty-five cows were studied over a period of 4 years. Fifteen of these cows remained free from infection and 3 were infected throughout the entire course of the investigation, while 17 became infected during the period in which the observations were made. Weekly quarter samples of fore-milk were secured from each cow and examined for presence of mastitis streptococci, the number of leucocytes per cc., reaction to brom thymol blue,

and the macroscopic appearance of the milk. Daily total milk and milk fat production records also were noted for each cow.

Infections of the udder did not affect production as reflected by the persistency in production when the only evidence of infection secured was the presence of mastitis streptococci or more than 500,000 leucocytes per cc. in the fore-milk. No appreciable effect upon production could be found until the infection was sufficiently advanced for the milk to be abnormal in physical appearance.

Latent and chronic mastitis when confined to one quarter did not significantly affect production. When such an infection involved three or more quarters significant effects could be noted. The progressive nature of the infection causes it eventually to become sufficiently advanced to affect production. Advanced infections when confined to one quarter did not affect production until the condition became active and the milk was abnormal in appearance.

The percentage of milk fat was not affected independently of the total production. Total and milk fat production trends responded similarly to the effects of infection. It was also found that slight infections as evidenced by presence of mastitis streptococci in the fore-milk or more than 500,000 leucocytes per cc. did not affect production materially.

It is concluded that streptococcal infections of the udder must be sufficiently advanced to make the milk alkaline to brom thymol blue or to show evidences of infection by changes in its physical appearance before a material effect can be noted on production of milk or milk fat.

#### Authors' Abstract.

#### 11. Mastitis. VIII. The Use of a Specially Prepared Vaccine in an Attempt to Control Bovine Mastitis. G. J. HUCKER AND PAUL ARNE HANSEN, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Techn. Bull. No. 245. July, 1937.

A study was made of the possible prophylactic and therapeutic action of a vaccine prepared from stock and freshly isolated herd strains of *Streptococcus agalactiae* Lehmann and Neumann. Injections of milk were used in connection with the vaccines.

One hundred two animals in four herds were studied over a period of 2 years, 45 being vaccinated and 57 being retained as untreated controls.

Weekly quarter samples were secured for 4 to 7 weeks prior to vaccination and weekly quarter samples for 3 months and monthly quarter samples for approximately 14 months subsequent to vaccination were examined in the laboratory for presence of mastitis streptococci, number of leucocytes per cc., reaction to brom thymol blue, and physical appearance of the milk.

It is concluded that vaccines prepared from stock and freshly isolated strains of *Streptococcus agalactiae* give no evidence of increasing the resistance of dairy cattle to mastitis.

Similar vaccines were found to have little or no therapeutic action in the treatment of latent and chronic udder infections. Neither the prophylactic nor therapeutic action of this vaccine was stimulated by the use of simultaneous intramuscular injections of milk.

Authors' Abstract.

**12. Mastitis. IX. The Maintenance of a Herd Free from Mastitis.** G. J. HUCKER AND E. S. HARRISON, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Techn. Bull. No. 246. July, 1937.

Three experimental herds which included a total of 271 cows were studied over a period of 3 years. Weekly quarter samples of fore-milk were examined for presence of mastitis streptococci, number of leucocytes per cc., reaction to brom thymol blue, and physical appearance of the milk.

One of the experimental herds, when the studies were inaugurated, contained no animals which discharged streptococci demonstrable by any of the methods used; another contained a moderate number; while the third contained a large percentage of infected individuals. The herd with the moderate amount of infection was divided into two sections. In one section the cows were milked in order of infection while in the other no effort was made to isolate or segregate the infected individuals.

Under the conditions studied it was impossible to maintain a herd free from cows which discharged mastitis streptococci in the milk. The percentage of new infections as evidenced by the appearance of mastitis streptococci in the fore-milk was proportionately decreased as the amount of infection in the herd decreased. In the herd which was relatively free from infection when the studies were inaugurated, only 11 per cent of the total cows developed an infection, while 30 per cent of the cows in the remaining two herds developed an infection as evidenced by mastitis streptococci in the milk.

It proved possible to maintain a herd relatively free from cows which produced abnormal milk.

Thirty-six per cent of all heifers from the experimental herds discharged mastitis streptococci in the fore-milk during the first week of lactation. The condition of the udder of the dam did not appear to affect the amount of infection in the heifer. A relationship was found, however, between the amount of infection in the parent herd and the incidence of infection in first-calf heifers.

Authors' Abstract.

**13. Chronic Mastitis.** G. J. HUCKER AND P. ARNE HANSEN, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Circular No. 147. Oct., 1936.

A discussion of chronic mastitis, its causes, detection and practical suggestions for its control.

T.S.S.

**14. A Study of Milk-borne Epidemics.** PAUL B. BROOKS, State Dept. of Health, Albany, N. Y. J. of Milk Techn., 2: 168-174. 1939.

A study of milk-borne epidemics over a 22 year period in New York State, exclusive of New York City, indicates that the majority of outbreaks originated on the farm. Some specific outbreaks are cited giving causes found. The value of pasteurization as a protection is emphasized.

L.H.B.

## FOOD VALUE OF DAIRY PRODUCTS

- 15. The Nutritional Properties of Milk.** C. P. SEGARD, Wis. Alumni Research Foundation, Madison, Wis. *J. of Milk Techn., 2: 249-251.* 1939.

A discussion is given of some of the food factors found in milk and their nutritional importance.

L.H.B.

## ICE CREAM

- 16. The Bacteriological Quality of the Ice Cream Supply for a Small City.** M. W. YALE AND R. C. HICKEY, N. Y. State Agr. Exp. Sta., Geneva, N. Y. *Techn. Bull. No. 248.* Sept., 1937.

With the exception of three or four of the larger cities in New York State, but little work is being done by municipalities on ice cream sanitation, and knowledge in respect to sanitary quality of ice cream and ice cream ingredients is quite inadequate.

A bacteriological study was made of the ice cream supply for a small city since it was believed that the results would be of general interest and applicable to the situation in many other municipalities.

Both total and coliform counts were determined for 77 process samples from 7 local plants, 137 ice cream samples from 12 retail stores, and 36 dipper water samples from 18 establishments. Process samples demonstrated that either gelatin or color was excessively contaminated at four plants and cream at one plant. Freezing equipment was in poor sanitary condition at two plants.

Coliform counts were more sensitive than either standard nutrient agar or tryptone agar counts in revealing contamination by ice cream dippers and dipper waters which were in poor sanitary condition in the majority of instances.

Three of the 12 manufacturers had all standard agar plate counts of store samples of vanilla ice cream under 100,000 per gram. Eight manufacturers had an average (logarithmic) standard plate count under 100,000 per gram. The average count of 112 store samples was 59,800 per gram, a higher average than usually found in cities and states where bacteriological control is exercised. In the case of 9 of the 12 manufacturers, there had been no previous bacteriological control of their product.

Authors' Abstract.

- 17. General Retail Store Management.** RORERT SUTTLE. *Ice Cream Trade J., 35: 10, 70.* 1939.

This is the seventh of a series of articles on retail store management. Attention is directed to the importance of an organized personnel with a direct line of authority from the general manager to the store supervisor, to the store manager, and to the counter salesman. The store supervisor should perform the following duties.

- (a) Inventory store periodically.
- (b) Check and analyze the store reports and payrolls.
- (c) Inventory periodically the store equipment.
- (d) Make general inspection of store.

W.H.M.

**18. Egg Solids.** M. A. WIDLAND AND M. J. MACK. *Ice Cream Trade J.*, 35: 10, 21. 1939.

The analysis of 7 samples of egg yolk powder showed that the fat varied from 30.66 to 61.75 per cent, yolk solids from 33.06 to 90.09, moisture 3.85 to 6.15, protein from 27.56 to 42, and pH from 5.44 to 6.10.

Fat determinations were made by using a modification of the Mojonnier method. One gram of powder was weighed in a butter boat and placed in a dry extraction flask. Ten cc. of alcohol was added and mixed thoroughly. Next 25 cc. of ethyl ether was added and shaken, followed by 25 cc. of petroleum ether and 2 or 3 cc. of water and thoroughly shaken. The usual Mojonnier procedure was followed from this point.

The moisture was determined by the toluene-distillation method now recommended for milk powder. Protein determinations were made by the Kjeldahl method. The pH determinations were made with a Leeds-Northrup potentiometer. The per cent of egg yolk solids present was determined on the basis of the lipoid content using Perlman's method (1933 Ann. Rept., p. 131, N. Y. S. Dept. of Agriculture and Markets).

The egg powders were used in ice cream and the following observations made. Ice cream containing egg powder was firm when drawn from the freezer, withstood heat shocking well, and had a smooth compact appearance relatively free from visible air cells. Flavor improvement is dependent on the quality of egg products used. Eggs also improved the texture of the ice cream. The egg powder blends appear to be of value to the extent that they contain egg yolk solids. In order that a manufacturer may comply with standards for egg content of custards it is desirable to know the composition of the egg product used.

W.H.M.

**19. Cost Control.** LOUIS M. KESSLER. *Ice Cream Trade J.*, 35: 9, 10. 1939.

Cost accounting may be looked upon as an effective tool which will help management, reduce costs, eliminate wastes and inefficiencies, and form a basis for critically analyzing current costs by comparing them with estimates previously set up to indicate what, in the light of good judgment, costs should

be at the current volume of production. In almost any system one will find the three-fold break down into materials, direct labor, and overhead.

Effective material control may be maintained by keeping the following records: mix and pasteurizing reports; thermometer readings; freezing room reports showing quantities of mix frozen and analysis of overrun; hardening room reports showing quantities in and out; a check system for products out and in by delivery trucks.

Adequate maintenance is necessary to give information for entries in the general accounts, for analysis and distributions, and for social security requirements. Time keeping and the handling of payrolls should be kept separate for purpose of internal check.

The control of overhead began with a break-down of expenses into these fixed and variable elements. Fixed expenses are those which are inherent in the capacity to produce. They vary little with changes in volume. Variable expenses are those which are incurred as operations begin and which vary more or less directly with activity.

Expense control requires the preparation of a flexible budget with provision for adjustment to conditions of actual activity. A hypothetical case is presented by the author to show how the flexible budget works. Control of costs can be made effective by watching costs as they come in to see that too many unexpected and unwelcome guests do not arrive. This is more effective than mere standing at the exit to place a price tag of so much per gallon on all products as they go out.

W.H.M.

**20. Plant Records.** PERRY E. PIPER. *Ice Cream Trade J.*, 35: 10, 35. 1939.

The author suggests that mix formulas be kept on small vari-colored shipping tags as a time saver for the mix department. He suggests the testing and standardizing of each mix so that the ice cream will meet legal requirements. A standardization chart and explanations for standardizing mixes are presented in detail. The importance of keeping plant records and maintaining a laboratory for plant control are emphasized.

W.H.M.

**21. Quick Frozen Foods, The Trend—The Reasons.** ANONYMOUS. *Ice Cream Trade J.*, 35: 9, 15. 1939.

This timely article presents the attitude of several ice cream manufacturers on the frosted food industry. Many feel that it is an opportunity for winter as well as summer profit at comparatively little additional overhead.

W.H.M.

**22. Let's Face the Facts on Frozen Foods.** CHARLES Q. SHERMAN. *Ice Cream Trade J.*, 36: 8, 8. 1939.

This article points out some of the pitfalls ice cream manufacturers should

avoid if they are to succeed in the frosted food industry. Dealers should not be exploited by selling them equipment which is not needed, rather they should be shown how to care for their equipment and the products which they sell from it.

W.H.M.

**23. How to Figure Overrun in Ice Cream.** B. I. MASUROVSKY. *Ice Cream Trade J.*, 38: 8, 39. 1939.

Two examples of how to figure overrun in fruit ice cream are presented. Assuming that 600 gallons of ice cream mix were used to manufacture strawberry ice cream, and 60 gallons of strawberry fruit and juice were incorporated in the finished ice cream—how much overrun was obtained when the yield was 1200 gallons of strawberry ice cream?

**Example I:** Solution: (1) 600 plus 60 equals 660 gallons of basic material (ice cream mix plus fruit).

(2) 1200 minus 660 equals 540 gallons overrun.

$$(3) \frac{540 \times 100}{660} = 81.8\% \text{ overrun.}$$

**Proof:** Step I 660 gallons of basic material weighs  $660 \times 9 = 5940$  lbs.

Step II Since 1200 gallons of finished strawberry ice cream weigh 5940 lbs., the weight per gallon of this ice cream is 4.95 lbs.

Step III Overrun by weight in this case is

$$9 - 4.95 = 4.05 \text{ or } \frac{4.05 \times 100}{4.95} = 81.8\% \text{ (overrun).}$$

**Example II.** Step I 1200 gallons of ice cream less the 60 gallons of strawberries would give 1140 gallons as the actual figure to be used in calculating the overrun due to the ice cream mix only.

Step II 1140 minus 600 equals 540 gallons of overrun.

$$\text{Step III } \frac{540 \times 100}{600} = 90\% \text{ overrun.}$$

W.H.M.

**24. Bacteria of the Colon-Aerogenes Group on Nut Meats.** MORRIS OSTROLENK AND A. C. HUNTER, Food and Drug Adm., U. S. D. A., Washington, D. C. *Food Research*, 4: 5, 453. Sept.-Oct., 1939.

Nut meats in the unbroken shell are free of the coliform bacteria. Various nut meats purchased on the retail market contained *E. coli* in from 4 to 45 per cent of the samples. Various nut meats obtained from domestic shelling plants contained the organism in from 6 to 68 per cent of the samples and imported nut meats in from 14 to 62 per cent of the samples. Five hundred and forty-eight samples of nut meats representing 11 varieties were examined. When large numbers of *E. coli* are present correspondingly large numbers

of *Arobacter aerogenes* and intermediates are found. Artificially contaminated nut meats stored at room temperatures contained viable *E. coli* organisms for approximately 68 days.

F.J.D.

**25. Preparation and Use of Low-Lactose Milk.** P. H. TRACY AND W. J. CORBETT, Dept. of Dairy Husbandry, Univ. of Ill., Urbana, Ill. Food Research 4: 5, 493. Sept.-Oct., 1939.

This report is similar to that published by Corbett (Ice Cream Review 22: 6: 34, Jan. 1939) and reviewed as Abstract 189, J. Dairy Sc. 22: 4, A77, 1939.

F.J.D.

**26. Trends in Ice Cream Costs.** Int. Ass'n of Ice Cream Mfrs., Washington, D. C. Special Bull. No. 62. Oct., 1939.

This bulletin contains an analysis of ice cream expenses, based on data for 1938. The data show that four expense classifications make up over three-fourths of the total expense: first, products, 39.05 per cent; second, labor, 22.79 per cent; third, supplies, 9.41 per cent; and fourth, depreciation, 6.85 per cent. Products cost for 1938 was at the lowest level in three years.

The trend in costs is given for the period 1934 to 1938, inclusive. Over this period, the products cost increased yearly, until 1938 when a decrease occurred. Manufacturing, delivery, selling and total costs were high in 1934 and 1935, lower in 1936 and 1937, and in 1938 increased somewhat toward the 1934-1935 level. The main reason for these changes in costs is the variation in the volume of ice cream produced.

Another interesting fact is the phenomenal growth of specialties. In 1925, 3,082,485 gallons of ice cream were sold as cups, specialties, ice cream on a stick, etc. In 1937 this had increased more than ten times, or to 33,940,094 gallons.

M.J.M.

**27. Report of Committee on Laboratory Methods.** A. H. ROBERTSON, State Dept. of Health, Albany, N. Y. J. of Milk Techn., 2: 184-187. 1939.

It was deemed unwise to adopt officially any of the Babcock modifications for testing ice cream for fat. There is, however, a need for a rapid sorting test, whereby all samples could be examined and those approaching, or below, the statutory standards could then be further examined by the official Roes-Gottlieb method.

Three tests are listed for this purpose, as follows: 1. Pennsylvania, or Doan, 2. Fucoma, or Gerber, 3. Illinois, or Garrett-Overman.

L.H.B.

**28. Report of Committee on Ice Cream Sanitation.** F. W. FABIAN, Mich. State College, East Lansing, Mich. J. of Milk Techn., 2: 193-196. 1939.

It is the committee's opinion that frosted malted milk, or any other similar frozen dessert should be labeled imitation ice cream.

Although different methods for controlling overrun in ice cream are in use in different states, a stipulation of the minimum weight per gallon in terms of food solids per gallon (1.6 lbs. food solids per gal.) seems to be the method most generally adopted.

The same sanitary requirements for paper containers in the milk industry should also apply for those used for ice cream.

Twelve states now have maximum bacterial standards for ice cream ranging from 75,000 to 500,000 per gram or cubic centimeter. L.H.B.

**29. A Note on Ice Lump Formation in Ice Cream Frozen in Continuous Freezers.** DAVID LEVOWITZ, New Jersey Dairy Laboratories, New Brunswick, N. J. *J. of Milk Techn.,* 2: 188-190. 1939.

It was found that ice cream frozen in continuous freezers sometimes contains small lumps of ice. This was found to be true when machines were adjusted for low overruns. As overrun was lowered, the ice cream became more and more moist, and finally ice lumps appeared.

In one plant the mix gave a perfectly dry surfaced ice cream down to 90 per cent overrun. Below that it became increasingly moist down to 60 per cent where small ice lumps became noticeable, becoming increasingly large as overrun was reduced. When a portion of this ice cream was diverted through the fruit injector assembly the ice cream drawn from the fruit feeder at 90 per cent overrun showed small ice lumps, which became increasingly large as overrun was decreased. At 50 per cent overrun ice lumps were as large as puffed rice grains.

The total solid content of the mix is a factor. Increasing the total solids content of a specific mix in any manner delayed the appearance of ice lumps.

L.H.B.

**30. Multi-Stage Ammonia Compression Systems for Low Temperature Work.** RALPH V. GRAYSON, Quick Freezing, Houston, Texas, AND H. L. FISCHER, Vilter Mfg. Co., Houston, Tex. *Ice Cream Refrig.,* 95: 2, 80.

The authors point out the advantages of using multi-stage ammonia compression systems in an ice cream plant. The possibility of carbon deposits in the cylinder head is greatly reduced. Machines will last longer and upkeep is less. The two stage machine requires from 25 to 30 per cent less power than a single stage machine of the same capacity. Where extremely low temperatures are desired a three stage machine may have advantages. Where multi-stage machines are used inter-coolers should be installed. For quick freezing plants the refrigeration plant costs per unit capacity become less as the size of the plant increases. A three stage refrigerating installation for a

plant of 500 lbs. hourly freezing capacity is \$34 per pound. A 1000 lb. plant costs \$24 per pound. A 1500 lb. plant costs \$20 per pound. A 2000 lb. plant costs \$19 per pound hourly capacity. L.C.T.

## MILK

**31. Comparative Fairness of Single Can and Weigh Vat Samples of Milk for Bacterial Counts as a Basis of Premium Payments to Grade A Dairymen.** M. W. YALE AND ROBERT S. BREED, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Techn. Bull. 673. July, 1936.

In a comparative study of the fairness of single can and weigh vat samples for bacterial counts as a basis of premium payments to dairymen, 1,100 samples were collected from 178 dairies at three grade A plants at Cortland and Homer, N. Y., in December, 1934. In a second study in April, 1935, 197 samples were taken from 49 dairies at one of the above plants.

Premiums will occasionally although rarely, be lost due to contamination of the vat sample with milk from a preceding dairy. If no cans of milk with a bacterial count exceeding 100,000 per cc. were received at grade A plants, contamination from residual milk would almost never result in premium losses. Whether or not a change in the system of sampling would affect total premium returns to grade A dairymen could not be determined from the studies, but the results indicated that the vat sample does not favor either the producer or the milk company.

Since the weigh vat sample is much more representative than a single can sample and since no other practical method of taking composite samples of grade A dairies exists at present, vat sampling is a desirable procedure.

### Authors' Abstract

**32. The Individuality of the Solids-Not-Fat of Milk.** FOLKE JARL. Zschr. f. Tierzücht. u. Züchtungsbiol. 43: 3, 350-361. 1939.

The data in Illinois Agricultural Experiment Station Bulletin No. 325 when analyzed show that significant differences exist between different cows in the content of protein and also in the content of lactose in their milk, even after allowance is made for the correlation of those with fat percentage. There were also individual differences in the regression of these constituents on fat percentage. There was a slight negative correlation between the regression of protein on fat and the regression of lactose on fat. J.L.I.

**33. Annual Committee Reports.** Association Bull. Intern. Assoc. Milk Dealers. 32nd year: No. 1, pp. 1-35. 1939. •

Activities of the 1938-1939 year are reported by the following committees, accident prevention, accounting advisory, laboratory methods, legislation, membership, milk definitions and standards, public relations, sales and advertising section, simplified practice and transportation. E.F.G.

**34. The Flavor of Milk.** N. Y. State Agr. Exp. Sta., Geneva, N. Y. Circular No. 167. August, 1936.

The flavors of milk are discussed under the following headings; "Flavors of milk as secreted by cows," and "Flavors which may develop in milk." A list of points to observe to insure fine-flavored milk are appended. T.S.S.

**35. Measuring the Bacteriological Quality of Milk.** C. K. JOHNS, Science Service, Dominion Dept. of Agriculture, Ottawa, Ontario, Canada. J. of Milk Techn., 2: 175-180. 1939.

The value of the various tests for determining the bacterial content of milk are discussed. It is pointed out that no one test will give all the information desired.

The plate count even when using a more suitable medium or lower incubation temperature will not indicate the true number of bacteria present in milk.

The methylene blue reduction test and the resazurin test have the advantage of rapidly detecting poor quality milks. They are both sensitive to the state of activity of the organisms present, but they do not indicate the source of the contamination. For this purpose the direct microscopic examination excels.

L.H.B.

**36. Educational Methods in Relation to Milk Sanitation.** H. S. ADAMS, Dept. of Health, Flint, Mich. J. of Milk Techn., 2: 162-167. 1939.

Some educational methods which have been found valuable and effective in milk control work are cited.

L.H.B.

**37. Testing of Bottle-Washing Solutions.** C. M. MOORE, The Diversey Corporation, Chicago, Ill. J. of Milk Techn., 2: 227-235. 1939.

Five methods are sometimes used for testing the strength of bottle washing solutions. They are hydrometer, total alkalinity, free caustic, pH, and conductivity tests.

When measuring the relation of strength of solution to germicidal action, the test for free caustic has the greatest value.

Tests for the germicidal efficiency of alkaline materials on different organisms at different temperatures are reported.

L.H.B.

**38. A Method for Checking the Holding Time in Short Time High Temperature Pasteurizers.** D. M. ROGER, Laboratory Dept., The Borden Farm Products Co., Brooklyn, N. Y. J. of Milk Techn., 2: 191-192. 1939.

A simple and accurate method for checking the holding time of a short time high temperature pasteurizer is to inject a saturated sodium chloride

solution into a stream of water entering the holding chamber. At this point a pair of electrodes connected with a circuit joining dry cells and a microammeter are located. Another pair of electrodes connected in a similar manner are also located at the outlet of the holding chamber. As the salt solution passes these electrodes, there is a sharp deflection of the ammeters. A stop watch is started when the first ammeter is deflected and stopped when the second ammeter is deflected; thus the actual holding time is easily checked. The hookup is illustrated by a diagram.

L.H.B.

**39. Pasteurized Cream Production Coordinated with Simultaneous Milk**

**Processing.** W. B. PALMER, Milk Inspection Association of the Oranges and Maplewood, N. J. *J. of Milk Techn.,* 2: 212-214. 1939.

Cream separated from properly pasteurized milk which has been properly handled to prevent contamination is equally as good as that pasteurized subsequent to separation; and less equipment and expense are involved.

A system is described whereby pasteurized milk may be by-passed to the separator when bottling pints and half-pints without necessitating a reduction of the flow from the vat.

A table giving bacterial counts of the cream and of the milk from which it was separated is presented.

L.H.B.

**40. The Use of the Phosphatase Test by New York City.** JOHN L. RICE, Commissioner of Health, New York City. *J. of Milk Techn.,* 2: 181-183. 1939.

The modified phosphatase test has proven to be of inestimable value in controlling the efficiency of pasteurization.

This test was adopted as a routine procedure in the New York City Health Department in March, 1937, and more than 100,000 determinations have been made.

When first used about 1.5 per cent of all samples showed evidence of gross irregularities in pasteurization. Within a few months, such irregularities were greatly reduced, and during the past year none have been found.

During the first three months that the tests were routinely used 6.6 per cent of the samples showed slight irregularities in pasteurization. At present minor irregularities are found in only about 2 per cent of the samples, and most of these are found in cream samples.

L.H.B.

**41. A Comparison of Plate Counts of Raw Milk on the Old Standard Nutrient Agar and on the New Tryptone-Glucose-Extract-Milk Agar.** C. A. ABELE AND S. R. DAMON, Ala. State Dept. of Health, Montgomery, Ala. *J. of Milk Techn.,* 2: 222-226. 1939.

Results obtained on 1,000 samples of raw milk, slightly over 50 per cent of which were of raw milk distributed at retail and the remainder of milk intended for pasteurizing, indicated that it was impossible to approximate the percentage of deviation in counts obtained on the two mediums.

There was an increase in count on the new medium over the old in 70.4 per cent of the cases; 7.0 per cent remained unchanged, and in 22.6 per cent there was a decrease.

The number of instances in which the higher count obtained on the new medium would have effected the grade of retail raw milk was only 7.4 per cent and of milk intended for pasteurization was only 2.6 per cent. About one-third of these retail samples and nearly two-thirds of these samples intended for pasteurization were very near the limit on the old medium.

The limits used were those set by the U.S.P.H. ordinance of 50,000 for grade A raw milk and 200,000 for raw milk intended for pasteurization in the upper grade of pasteurized milk.

Results do not indicate a need for changing plate count limits now in use.

L.H.B.

#### 42. Application of Resazurin Test in Determining Quality of Raw Milk

and Cream. M. A. COLLINS, L. M. WHITE, W. H. TURNER, JR. AND J. R. RICE, Quality Control Div., United Farmers Coop. Creamery Assn., Boston, Mass. and Morrisville, Vt. *J. of Milk Techn.*, 2: 236-244. 1939.

Tests indicate that the resazurin test can be applied at different laboratories with uniform results when methods are standardized. It was indicated that fairly uniform agreement could be obtained by several workers in reading the tests at an intermediate pink color (pronounced pink but not a vivid pink) and thus make the test more useful.

Comparative tests were made on raw patrons' milk, tank car shipments of milk and on vats of raw cream using the resazurin test to pink and to white, the methylene blue test, the direct microscopic count and the agar plate count.

One ml. of 0.005 per cent resazurin solution was mixed with 10.0 ml. of milk or cream.

The reduction time for resazurin to white and methylene blue to white were approximately equal. The reduction time for resazurin to pink was about two-thirds the reduction time of methylene blue on tank car shipments. On patrons' milk it was approximately one-half the methylene blue reduction time. There was a high agreement between the resazurin test to pink and the methylene blue test in selecting both poor and good quality milks. A resazurin test to pink in three hours is equivalent to a 5.5 hour methylene blue test.

Patrons' milk having a resazurin test to pink in three hours or more was

found to have a bacterial count of less than 400,000 per ml. Five to six hours indicate less than 100,000 per ml. L.H.B.

**43. Requirements of Farm Electric Milk Coolers.** JOHN E. NICHOLAS, Penn. State College. *Ice and Refrig.*, 95: 5, 324.

This paper presents a discussion of the types and construction as well as performance of various farm electric milk coolers. The author summarizes the principal requirements of a farm electric milk cooler as follows:—

1. Cool the milk rapidly.
2. Cool the milk uniformly.
3. Provide water agitation for rapidity and uniformity of cooling.
4. All operating features should be automatic.

#### MISCELLANEOUS

**44. A Portable Calorimeter for Small Compressors.** D. D. WILE. *Ice Cream Trade J.*, 36: 8, 12. 1939.

A portable calorimeter for small compressors, which overcomes many of the disadvantages of other equipment used for this purpose, is described. The instruments are mounted on a frame which rests on casters for easy portability. The various instruments in the panel are:

1. Suction gage.
2. Discharge gage.
3. Watthour meter for motor.
4. Watthour meter for heater.
5. Pressure gage for secondary refrigerant.
6. Pressure control for secondary refrigerant.
7. Sight glass for liquid feed line.
8. Control switches and plug receptacles.
9. Mercury column for suction pressure.
10. Thermocouple jack.
11. Adjusting knob for expansion valve.

W.H.M.

**45. Highway Transportation Re-makes America.** National Highway Users Conference, National Press Bldg., Washington, D. C. Sept., 1939. Also available from the Int. Ass'n of Ice Cream Mfrs., 1105 Barr Bldg., Washington, D. C.

This comprehensive review of the use of highways and the problems now existing in highway development should be of interest to those engaged in the transportation of dairy products. M.J.M.

**46. Accounting for Fixed Assets.** O'NEAL M. JOHNSON, Int. Ass'n of Ice Cream Mfrs., Washington, D. C. Special Bull. No. 61. Sept., 1939.

This is a treatise explaining why fixed asset accounting is necessary and how it may be accomplished. A supplement is included which deals with depreciation accounting for income tax purposes.

M.J.M.

**47. Refrigerated Food Locker Plants.** W. H. Motz, Jefferson Ice Company, Chicago, Ill. *Ice and Refrig.*, 95: 6, 446.

It is estimated that there are about 2,800 locker plants in operation with 50 new ones added monthly. There are 1,000,000 lockers in use with each locker having an annual storage usage of 500 pounds. The following facilities are advisable: Receiving room, chill room maintained at 32° to 36° F and 85 to 90 per cent relative humidity, holding or aging room maintained at 32° to 36° F and 80 to 85 per cent relative humidity, freezer room at -5° to -15° F and locker room at 5° F. Under ordinary conditions products may be stored as follows: Fresh pork, 3 to 4 months; beef, 6 months; lamb, 6 months; poultry, 6 months; vegetables, 3 to 4 months; fruits in syrup, 12 months. Layouts of plants are included in the discussion. Data on cost of construction is included. Charts on operating costs are presented.

L.C.T.

**48. The Cold Storage Locker Plant—Features of Design and Heat Load Analysis.** A. G. VOGEL, Renipe Co., Chicago, Ill. *Ice and Refrig.*, 95: 6, 444.

Rooms are most satisfactory when 10 feet high. Lockers are usually 17" high × 20" wide × 30" long and are arranged in tiers five lockers high with aisles 3 feet wide, and 2 or 3 inches space between lockers and walls for circulation. This is equivalent to approximately 8½ square feet of floor area per tier of lockers. The required heat load is equivalent to about 187 B.T.U. per hour for each stand of lockers. To maintain the locker room at 10° F with suction gas at -10° F there should be 10½ lineal feet of 1½" standard wrought steel pipe. The freezing room area should equal at least 10 per cent of locker room area. The heat load is about 31 B.T.U. per locker. If one half of the piping is used as shelving the number of lineal feet may be reduced 20 per cent. The area of the chill room should not be less than 25 per cent of the locker room. The heat load is equal to about 22 B.T.U. per locker. Unit coolers are best for this room and should be operated at air velocities which do not exceed 30 feet per minute. All heat loads noted are for machine operation of 16 hours out of 24. All rooms should be insulated with the equivalent of 8" of cork.

L.C.T.

**49. Some Indicating and Recording Instruments Indispensable in Refrigeration.** CHARLES H. HERTER. *Ice and Refrig.*, 95: 5, 326.

The author lists the types of instruments and their location and interpretation in connection with refrigeration systems. Pressure gauges should

be checked yearly for accuracy. If the amount of water used and the temperature rise in the condensing water is known the refrigerating capacity can be estimated by assuming that 250 B.T.U. per minute are absorbed per ton capacity. The temperature of liquefaction should be within 5° F of that of the water leaving the condenser. The temperature of the gas entering the compressor as well as its discharge temperature should be determined. All rooms should be provided with thermometers. Several types of humidity measuring instruments are described. Where installations are large enough recording equipment is recommended.

L.C.T.

**50. The Rise of Cold Storage Lockers and Locker Plants.** P. EDWIN THOMAS. *Ice and Refrig.*, 95: 4, 289.

The author presents a brief outline of the development of the locker system and surveys the possibilities and probable future expansion. A very able discussion of the advantages of the cold storage locker system is included. A general plan of a locker plant is given. A table of cost of operation for a 320 locker plant is included and is placed at \$6,077.80 per year when operated independently of any other business. This includes, of course, all servicing and processing charges; so that the actual locker rental need not exceed \$10.00 or \$12.00 per year.

L.C.T.

**51. Stack Effect—The Missing Variable of Bunker and Coil Design.** HAL WEIR MCPIERSON. *Ice and Refrig.*, 95: 2, 95.

Data comparing stack coils and bunkers for use in refrigeration and air conditioning is given. The author likewise includes considerable information from a technical standpoint concerning the theory of heat exchange.

L.C.T.

**52. Absorption Refrigeration.** J. C. BERTSCH. *Ice and Refrig.*, 95: 4, 223.

The author traces briefly the patent history of absorption refrigeration system and presents diagrams of several of the leading makes, together with a description of their operation.

L.C.T.

**53. Automatic Controls—Results Obtained from Their Modern Application.** W. E. ZIEBER, York Ice Machinery Corp., York, Pa. *Ice and Refrig.*, 94: 3, 114.

Various controls are classified and described. Three systems are mentioned, namely, direct acting, air operated, and electrically operated. A number of application diagrams are included showing the location of automatic devices.

L.C.T.



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Baltzer, A. C.	England, C. W.	Knight, D.	Reid, W. H. E.
Barkman, J. O.	Erb, J. H.	Krauss, W. E.	Richardson, G. A.
Bartlett, J. W.	Espe, D. L.	LeMaster, J. P.	Riddell, W. H.
Becker, R. B.	Fabian, F. W.	Leighton, A.	Schultz, E. N.
Bendixen, H. A.	Frayer, J. M.	Lindquist, H. G.	Sommer, H. H.
Bennett, F. W.	Frazier, W. C.	Locke, R. B.	Stark, C. N.
Bird, E. W.	Fuller, J. M.	Lucas, F. S.	Swope, W. D.
Burgwald, L. H.	Garrett, O. F.	Lush, J. L.	Tarassuk, N. P.
Burri, R.	Gelpi, A. J.	Mack, M. J.	Theophilus, D. R.
Brucekner, H. J.	Golding, N. S.	Macy, H.	Thomsen, L. C.
Burke, A. D.	Goss, E. F.	Mann, A. L.	Thurston, L. M.
Bushnell, L. D.	Greenbank, G. R.	Marquardt, J. C.	Totman, C. C.
Cannon, C. Y.	Gullickson, T. W.	Martin, W. H.	Trout, G. M.
Carpenter, D. C.	Guthrie, E. S.	Mead, S. W.	Tuckey, S. L.
Cave, H. W.	Hansen, Arne	Moore, L. A.	Webb, B. H.
Clevenger, W. L.	Herrington, B. L.	Morris, A. J.	Weckel, K. G.
Cole, W. C.	Herzer, F. H.	Mueller, W. S.	White, G. C.
Copeland, L.	Holdaway, C. W.	Nelson, D. H.	Wilbur, J. W.
Coulter, S. T.	Horrall, B. E.	Nelson, J. A.	Wilster, G.
Cunningham, O. C.	Huffman, C. F.	Overman, O. R.	Wylie, C. E.
Cunningham, W. S.			Yale, M. W.
Dahlberg, A. C.	Irvin, O. R.		

## JOURNALS

American Creamery and Poultry Produce Review	Journal of Genetics
American Journal of Diseases of Children	Journal of Infectious Diseases
American Journal of Physiology	Journal of London Chemical Society
American Journal of Public Health	Journal of Milk Technology
Archives of Pediatrics	Journal of Nutrition
Biochemical Journal	Journal of Pathology and Bacteriology
Biochemische Zeitschrift	Journal of Physical Chemistry
Canadian Dairy and Ice Cream Journal	Journal of Physiology
Canadian Public Health Journal	Kaeseindustrie
Certified Milk	Kolloid-Zeitschrift
Cornell Veterinarian	Lancet
Dairy Industries	Le Lait
Deutsche Molkerei Zeitung	Milchwirtschaftliche Forschungen
Endocrinology	Milchwirtschaftliche Zeitung
Food Industries	Milk Dealer
Food Manufacture	Milk Industry
Food Research	Milk Plant Monthly
Guernsey Breeders Journal	Molkerei Zeitung
Ice and Refrigeration	National Butter and Cheese Journal
Ice Cream Field	Pacific Dairy Review
Ice Cream Industry	Proceedings of Society of Animal Production
Ice Cream Review	Proceedings of Society of Experimental Biology and Medicine
Ice Cream Trade Journal	Scientific Agriculture
Industrial and Engineering Chemistry	Tierernährung
Jersey Bulletin	Tierzüchter
Journal of Agricultural Research	Trudy Vologodskogo Molochnogo Institut
Journal of Agricultural Science	Zeitschrift für Infektionskrankheiten Parasitäre Krankheiten und Hygiene der Haustiere
Journal of American Chemical Society	Zeitschrift für Physikalische Chemie, Abt. A and B
Journal of American Veterinary Medicine Association	Zeitschrift für Untersuchung der Lebensmittel
Journal of Bacteriology	Zeitschrift für Züchtung, Reihe B, Tierzüchtung und Zuchtbioologie
Journal of Biological Chemistry	Zentralblatt für Bakteriologie
Journal of Dairy Research	Züchtungskunde
Journal of Dairy Science	
Journal of Experimental Medicine	
Journal of General Physiology	
Journal of Heredity	

## SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland	Prussian Dairy Research Institute, Kiel, Germany
International Association of Ice Cream Manufacturers	State Agricultural Colleges and Experiment Stations
International Association of Milk Dealers	The Royal Technical College, Copenhagen, Denmark
National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

### BACTERIOLOGY

- 54. Results of Bacterial Plate Counts of Milk on Three Media and at Two Temperatures of Incubation.** C. A. ABELE, State Dept. of Public Health, Montgomery, Ala. *Am. J. Pub. Health, 29: 8, 821, 1939.*

The author, acting as referee of a committee on Standard Methods for the examination of milk and dairy products, analyzed data obtained by seven public health laboratories. A total of 335 milk and cream samples was plated on the old standard agar, tryptone-glucose-extract-skim milk agar (standard since July 1, 1939), and the American Association of Medical Milk Commissions agar, the plates were then incubated at 32° C. and 37° C.

The results of this study parallel those of other studies conducted, and corroborate the conclusions drawn therefrom. The use of the new T-G-E-M agar usually resulted in an increase over the plate count on the old standard nutrient agar and also, in general, yielded slightly higher counts than the American Association of Medical Milk Commissions agar.

Incubation at 32° C. in combination with the use of the modified agars, resulted in increases in plate count of considerably greater magnitude than were obtained by the use of these agars and incubation at 37° C.

M.W.Y.

### BUTTER

- 55. Handling Cream between Pasteurizer and Churn.** O. F. HUNZIKER. *Can. Dairy and Ice Cream J., 18: 8, 53, 1939.*

The more important factors which contribute to the development of oily-metallic flavor in butter are (1) high acid cream, (2) high fat cream, (3) high temperature of pasteurization, (4) prolonged holding after pasteurization and cooling, and (5) the presence of metallic salts. Overworking decreases deterioration due to bacteria but increases that deterioration due to chemical action such as oxidation. Excessive washing of butter washes out some of the desirable flavor and jeopardizes the keeping quality. Chlorination of the wash water to make it sterile is suggested. O.F.G.

- 56. Preserving Cream with Salt.** C. H. CASTELL AND E. H. GARRARD. *Can. Dairy and Ice Cream J., 18: 7, 19, 1939.*

The addition of 1 to 5 per cent by weight of salt to 35 per cent cream maintained at 77° F. had little or no effect but both No. 1 cream and accumulated cream to which 7 per cent salt was added and which was stored at various temperatures showed less acid production and better keeping

qualities than normal cream under the same conditions. The presence of 7 per cent salt considerably inhibited the growth of lactic acid bacteria and oxidizing types of organisms but had little effect on the total number of bacteria at the end of 8 days storage. Butter made from cream to which 7 per cent salt had been added showed a better flavor score than butter made from normal cream held at considerably lower temperatures. O.F.G.

### CHEESE

57. **Pasteurization of Milk for Cheesemaking.** J. G. DAVIS. Can. Dairy and Ice Cream J., 18: 8, 21, 1939.

Milk is pasteurized for cheesemaking for the following reasons: (1) to destroy fault-producing bacteria, (2) to give a more uniform product, and (3) to increase yield. The high-temperature short time method has the advantages of being a continuous process and requires little space. A temperature of 162° F. for 15 seconds is recommended. Ripening to a typical high flavor is slowed by pasteurization. Pasteurization does not become an incentive to use poor quality milk. Very few deleterious effects can be attributed to pasteurization if correctly done. O.F.G.

### CONCENTRATED AND DRY MILK: BY-PRODUCTS

58. **A New Name for Dry Milk Solids.** ANONYMOUS. Am. J. Pub. Health, 29: 10, 1155, 1939.

The concluding paragraph of this editorial states, "Because of the present wide use of dry milk solids and the unfortunate connotations of the words 'skim' or 'skimmed' in the public mind, it has been suggested that official designations of this product be changed from 'dried skimmed milk' to 'dry milk solids not over 1½ per cent fat', a definition which already has been adopted in two or three states. Since this terminology would permit of accurate and truthful labelling of a wholesome product, and would tend to remove the popular misconception about the real value of skimmed milk, the proposed new definition seems reasonable and deserves favorable consideration." M.W.Y

### DISEASE

59. **Second Report of the Use of Large Doses of Sulfanilamide in the Treatment of Chronic Streptococcal Mastitis.** W. T. MILLER, F. M. MURDOCK, AND J. O. HEISHMAN, Animal Disease Station, U. S. Bureau of Animal Ind., Beltsville, Md. J. Am. Vet. M. Assn., 95: 749, 140, Aug., 1939.

Each of three lactating cows received large doses of sulfanilamide for a period of six days in an attempt to cure chronic streptococcal mastitis. The streptococci were not removed permanently from the udders of the cows,

but temporary removal of these organisms from the milk was observed. The cows showed little ill effect from the treatment, except for a temporary decrease in milk production and some loss of weight. The authors state that sulfanilamide can hardly be considered of value in treatment of chronic mastitis caused by streptococci but observed that sulfanilamide appeared to have a transient inhibitory action on the streptococci in the udder, suggesting a possible use for it in the treatment of acute attacks of mastitis due to these organisms.

J.W.W.

#### 60. Outbreak of Staphylococcus Milk Poisoning in Pasteurized Milk.

JOHN F. HACKLER, Payne County Health Unit, Stillwater, Okla.  
Am. J. Pub. Health, 29: 11, 1247, 1939.

Epidemiological investigation revealed that all of the 29 cases had drunk varying amounts of milk produced by one local pasteurizing plant. "Potentially toxic staphylococci" were isolated from samples of milk causing illness and upon which the phosphatase test revealed adequate pasteurization. The author concludes, therefore, that contamination occurred after pasteurization and presumably during the processes of bottling and capping, which were done by hand.

M.W.Y.

#### 61. Tuberculous Infection Due to Milk. ANONYMOUS. Am. J. Pub. Health, 29: 10, 1154, 1939.

This editorial is based on an outbreak of an acute epidemic of tuberculosis in Horred, Sweden, which was traced to a single cow with tuberculosis of the udder. The herd was under a state organization whose object was the prevention of tuberculosis in cattle in an infectious form and the herd had regularly received veterinary inspection. It is clear that clinical examination of cattle to detect tuberculosis is not to be wholly depended upon and the necessity of pasteurization has been shown in a striking manner.

M.W.Y.

#### 62. A Practical Method of Herd Management to Combat Mastitis. G. E. DIX. Can. Dairy and Ice Cream J., 18: 7, 57, 1939.

This article describes the methods used on a grade A milk farm in eliminating mastitis from the herd. The predisposing causes of mastitis are listed as (1) chilling of the udder, (2) injuries to the udder, and (3) injections of the udder. The importance of a carefully controlled routine in milking is emphasized and specific procedures are given.

O.F.G.

#### 63. Chronic Mastitis of the Dairy Cow. E. G. HASTINGS, *et al.*, Committee on Bacteriological Problems. Assoc. Bull., Intern. Assoc. of Milk Dealers, 31st year: 14, 1-27, 1939.

This bulletin is a revision of a similar report published in 1936. Its purpose is to present such a summary of the present knowledge of this subject

as will help the milk dealer in his relations with producers and consumers of milk and with regulatory agencies to meet the various problems which mastitis presents. After a general statement with respect to the importance of mastitis the causes, incidence and effect of mastitis on milk production are discussed. The decrease in sugar, casein, soluble calcium and acidity are noted as well as the increase in the chloride content. In the fluid milk industry mastitis has an aesthetic significance which should not be lost sight of. In the cheese industry there is abnormal action toward rennet. Mastitis milk does not ordinarily constitute a serious health hazard. Standards by which the degree of severity of involvement may be judged are discussed, also the spread and diagnosis of the disease. An evaluation is made of the results of physical examination of the udder, strip cup, acidity tests, white blood cell counts and chloride tests as means of diagnosis. Methods for detection of streptococci are outlined. With respect to herd management some suggestions are given with the concluding advice that the herd owner will be wise to confine his efforts in combatting the disease to those procedures of herd management which are less expensive than medication or vaccination and probably more effective.

E.F.G.

## FOOD VALUE OF DAIRY PRODUCTS

### 64. Comparative Digestibility of Soft Curd Milks in Vitro. F. J. DOAN AND C. C. FLORA. Penn. Agr. Exp. Sta., Techn. Bull. 380, April, 1939.

The study was carried out primarily in an effort to obtain definite information relative to the accuracy of curd tension measurement as an index of the digestibility qualities of various types of soft curd milk. The study is important insofar as it deals with the altering of the coagulation process of cow's milk to obtain characteristics more nearly approaching those of breast milk.

The in-vitro method was compared with the in-vivo method in order to eliminate error as much as possible.

The results of this study were substantiated by rat feeding trials followed by post mortem examination of the digestive tracts.

It is concluded that digestibility of natural milk or pasteurized milk appears to be roughly in inverse proportion to curd tension.

Homogenization of milk lowers curd tension considerably but apparently improves digestibility very slightly, if at all.

The digestion qualities of trypsin treated milk appear to be somewhat better than would be anticipated from the curd tension value and the same statement may be made for citric acid treated milk where the acidification is not carried to the isoelectric range.

Heated milks are acted upon by trypsin at a more rapid rate than unheated milks.

Curd particle size apparently would be a more accurate index of the digestibility of milk and its suitability for use by infants than is curd tension.

F.J.D.

**65. Importance of Milk to Human Nutrition.** MARIE C. HARRINGTON,  
Dairy Council of St. Louis. Milk Dealer, 29: 1, 50-56, 1939.

The author shows the importance of milk to human nutrition in that it supplies protein, energy, vitamins A, B, C, D, and G, minerals, calcium and phosphorus.

C.J.B.

### ICE CREAM

**66. Outline of Activities for the Year 1939.** ROBERT C. HIBBEN. Int. Assn.  
of Ice Cream Mfrs., Washington, D. C., November, 1939.

This bulletin contains a resume of the activities of the International Association of Ice Cream Manufacturers for the past year. The work of the association largely centered around three subjects, namely: Government regulation (Federal and State); Inter-industry relations; and Association activities.

The association has actively worked for the industry to see that the Federal Food and Drug Act is acceptable to ice cream manufacturers. Other proposed acts or revisions of existing laws which might affect the industry have been closely followed.

Another phase of activity has been the dissemination of correct industry information to other industries, associations, and to the consumer.

The statistical and accounting bureau has studied taxation, accounting, has made expense comparisons, statistical surveys and has reported on trends in ice cream sales.

This bulletin also contains a report of the year's activities of the Ice Cream Merchandising Institute.

M.J.M.

**67. A Discussion of Chocolate Flavorings for Ice Cream.** G. R. A. MAY-BEE. Can. Dairy and Ice Cream J., 18: 7, 49. 1939.

The salt balance of the mix is affected by the type of cocoa used and since Dutch processed cocoas contain carbonates, the addition of these negative ions in the cocoa works in the direction of better whipping ability for the mix. If an untreated cocoa is to be used the pH must not be so low as to cause fat clumping or coagulation of protein; therefore, it should be above 6.0. If a treated cocoa is used, it should not have been overneutralized with alkali and the pH should not be above 7.0. Cocoa with a high fat content tends to impede overrun. The finer the cocoa is ground the deeper will be the color of the mix and the stronger will be the flavor.

O.F.G.

## MILK

- 68. Competition between Fresh Milk and Canned Milk.** LELAND SPENCER, Cornell University, Ithaca, N. Y. Assoc. Bull., Intern. Assoc. Milk Dealers, 32nd year, No. 3, 61-80, 1939.

In 1921 canned milk comprised 5 per cent of the milk consumed while by 1938 the proportion had increased to almost 9 per cent. Of all sections of the United States the largest per capita consumption of canned milk occurred in cities of the Rocky Mountain section and the smallest amount in the New England and North Central states. Of the total milk consumed by Negroes 19.1 per cent was canned milk, while Italian and Jewish peoples used 3.9 per cent and 4.3 per cent respectively in this form. The proportion of families using canned milk was much smaller in the groups where the incomes were large enough to permit considerable freedom of choice in making up the menu. The conclusion is reached that canned milk does tend to take the place of fresh milk and cream to a certain extent, but the families who use canned milk also consume a larger total of milk equivalent than they would if they were unable to buy canned milk at comparatively low prices. A remarkably large proportion of people use evaporated milk because of convenience or because they "like it better." Up to about 1922 a one pound can of evaporated milk retailed for nearly as much as a quart of fresh milk. In the past five years the retail price of fresh milk has averaged 14 per cent lower than during the 1925-29 period, but evaporated milk has been reduced 29 per cent during the same period. The extra price charged for fresh over evaporated milk has been split almost equally between payments to producer and dealers spread. High costs of labor for fresh milk distribution suggests urgent need for economy measures. Although it seems at times as if fluid milk prices have been at too high a premium over condensery milk, still it is not precisely known what is the excess cost of producing fluid milk. A constructive move is the present one to relate fluid milk prices more closely to those of the condensery and butter market. This article contains numerous tables, graphs and other factual material.

E.F.G.

- 69. Symposium—Effecting Economies in Plant Operations—Equipment.** GEO. W. WILSON, Kristoferson's Dairy, Seattle, Washington. Assoc. Bull., Intern. Assoc. Milk Dealers, 32nd year, No. 2, 45-50, 1939.

Successful experience is reported with V-belts on a vat, soaker washer, can and case washers, ammonia compressors and can conveyor. Three hundred pound type valves with long tapered hard metal seat and cone have saved greatly on hose lines, vat sterilizer and heating lines because of more positive action and longer life. A red hose has shown longer life than the

white hose. A fan operated evaporative condenser has reduced water use and the temperature of the ammonia going into receivers by 15 degrees. A well furnishing 50° F. water in place of the 70° F. water from city mains has fully paid for itself in six years. Many advantages are listed for no-roll churns. For washing powder a small scoop in the barrel replaces the large one. Special lubricants are used on places subjected to moisture and on conveyors. Straight line flow of products through the plant is recommended.

E.F.G.

**70. Refrigeration as Used with Pasteurization.** GEO. F. POPPENSIEK, Borden's Farm Products, New York City. Assoc. Bull., Intern. Assoc. Milk Dealers, 32nd year, No. 2, 51-53, 1939.

A practical discussion of the merits of sweet water, brine and direct expansion is presented. Sweet water has the advantages of being fool-proof and of little danger of contaminating the product, but lacks somewhat in flexibility in a large plant. Brine is flexible and does not corrode equipment if acid is checked once each month and the brine acidity or alkalinity adjusted to neutrality. Brine is best for plate type coolers. Two brine pumps are recommended; the second circulating 28° F. brine from the main circuit, passing it through the plates and back to the main circuit which might be at 25° F. In direct expansion systems a balanced load must be maintained if advantage is to be taken of potential savings in power costs.

E.F.G.

**71. Municipal Milk Plant of Wellington, New Zealand.** R. B. STOLTZ, Ohio State University, Columbus, Ohio. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year, No. 12, 295, 1939.

The reasons for the establishment of a municipal milk plant in Wellington, New Zealand, were a poor milk supply and the unwillingness of dealers to pasteurize. The quality of milk distributed by the plant was superior to most of the milk sold in New Zealand. It is a very modern and up-to-date plant and is better than the average in the United States. Many details with respect to procurement, processing, quality, delivery and costs are included in the discussion.

E.F.G.

**72. Feed Flavors and Practical Means of Controlling Them in Dairy Products.** C. E. WYLIE AND THOS. B. HARRISON, Dairy Department, University of Tennessee, Knoxville, Tenn. Milk Dealer, 29: 1, 132-134, 1939.

A brief discussion is given of some means of preventing feed flavors in milk.

C.J.B.

**73. Demand Growing for Milk Bottle Closures of Cover Cap Type.** ANONYMOUS. Milk Dealer, 29: 1, 82, 1939.

Increased demand for milk-bottle closures of cover cap type is shown by a list of municipalities that have passed ordinances governing the bottling and capping of milk with sanitary closures. C.J.B.

- 74. Similarity of the Oxidized Flavor from Three Sources.** D. H. NELSON AND C. D. DAHLE, Dairy Department, Pennsylvania State College. *Milk Dealer*, 29: 1, 62-66, 1939.

Following a brief review of the literature on oxidized flavor, the authors present data showing that spontaneous and copper-induced flavors are identical. C.J.B.

- 75. Bacteriological Control for Market Milk Plants.** R. G. SMITH. *Can. Dairy and Ice Cream J.*, 18: 7, 38, 1939.

The importance of competent laboratory service for the dairy operator is stressed if the dairyman wishes to be positive that he is supplying consumers with a safe and uniform product. The proper bacteriological methods to be used at the farm, in the dairy and in the laboratory are discussed. Proper administration of bacteriological control of milk tends to create a friendly spirit of cooperation between producer and processor. O.F.G.

- 76. Some Factors Affecting Milk and Cream Sales.** W. H. E. REID. *Can. Dairy and Ice Cream J.*, 18: 7, 23, 1939.

Homogenization changes the physical properties of milk and if carried out on unheated milk greatly accelerates the development of rancidity. The process retards the development of oxidized flavor but increases the susceptibility of milk toward sunlight flavor. Pasteurization of milk at increased temperatures decreases the length of the cream column (at 145° F. there was a decrease of 10.11 per cent). Undesirable flavors may be intensified by freezing of the milk and change in physical properties results. Agitation of cooled milk is likely to cause a decrease in creaming ability. If too complete creaming occurs a bluish appearance shows beneath the cream line. A high acidity, a high calcium and magnesium content, a low citrate and phosphate content, slow and complete freezing, slow thawing and partial churning are given as the causes of flakiness in market milk. The mottled appearance of the cream layer in bottled milk is not abnormal. O.F.G.

- 77. The General Economic Situation as Related to the Dairy Industry.** LELAND SPENCER, Cornell Univ., Ithaca, N. Y. *Assoc. Bull., Intern. Assoc. Milk Dealers*, 31st year: 10, 243, 1939.

The close relation of milk sales to business activity is shown. The widening spread between prices of fluid and manufacturing milk is noted and parallels drawn with other industries. The fact that retail prices and

dealers spread do not follow closely the variations in farm prices is explained by the fact that the dealer has better control of prices and that retail prices include a greater proportion of inflexible costs. Milk dealer profits fluctuate less from year to year than those of other industries but probably average about the same over longer periods. Many economic difficulties may be traced to inflation and deflation caused by wars and the milk dealer shares the ill effects with all business.

E.F.G.

- 78. Producer-Distributor Problems.** LOUIS J. TABER, The Nat'l Grange, Columbus, Ohio. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year: 12, 310, 1939.

This is a challenge to the dairy industry to find a way whereby the producer, distributor and consumer can sit around the table and by common sense American methods adjust their problems and when this is done the consumption of milk will be increased so tremendously that present problems will largely fade away and health, stamina and character will be immeasurably improved.

E.F.G.

- 79. New Developments in Milk Bottles.** J. F. WATSON, Borden Farm Products, New York City. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year: 12, 303, 1939.

The industry's annual bill for milk bottle replacements runs about \$14,000,000. In 1936, following a study by the Borden Company, the 22 oz. qt. bottle gradually came into use effecting an annual saving to the industry of three-quarters of a million dollars. This has also made possible lighter crates. If further reductions in the weight of the bottle are made it probably will be necessary to make a squat type bottle to attain strength. A square bottle has been used for orangeade and effected a 20 to 30 per cent saving in space, but is not as strong as the round bottle and more research work will need to be done before it will be a satisfactory milk container. A colored moving picture showed different types of bottles and closures and the handling of bottles in this country and in Europe.

E.F.G.

- 80. Demonstration of Proper Assembly of Fittings and Fabrication of Milk Lines.** E. N. MUZZY, Carnation Co., Seattle, Wash. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year: 13, 321, 1939.

An illustration shows the correct way to connect sanitary piping in various situations. This was an excellent demonstration with actual fittings. The demonstration was followed by extended discussion by W. D. Tiedeman and Geo. W. Putman.

E.F.G.

- 81. Short Time Pasteurization—A Sanitarian's View.** PAUL F. KRUEGER, Board of Health, Chicago, Ill. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year: 13, 334, 1939.

After extensive tests of four commercial installations in which some design changes were incorporated it was found that proper pasteurization temperatures could be maintained and proper holding times assured by the use of the high temperature short time equipment tested with the same degree of accuracy and safety as is possible with existing long time low temperature equipment. The temperature of 161° F. for 16 seconds had about the same effect on the cream line as 144° F. for 30 minutes. Plant operators have claimed that it is impossible for anyone to make a flavor distinction between Grade A raw and Grade A pasteurized when a high temperature short time system is used. From a bacteriological standpoint there is no increase in thermophyles in the pasteurized milk as compared with the raw milk. High counts were found to come from milk from farms, 90 to 95 per cent of which used milking machines. Lye treatment of milking machine tubes usually remedied the trouble caused by thermoduric organisms.

Pasteurizing milk at a temperature of 161° F. for 16 seconds in suitable apparatus equipped with proper controls may be considered as equally efficient as 144° F. for 30 minutes under similar conditions. E.F.G.

## 82. State Sanitary Control—Minimum and Maximum Requirements.

CHARLES McDONALD, Akron Health Dept., Akron, Ohio. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year: 13, 340, 1939.

Minimum regulations to be enacted by the state and maximum regulations passed and enforced by local health departments are given. Maximum regulations should be uniform without duplication of inspection service. Trained sanitarians should be employed in *all* sanitary milk control divisions, both state and municipal. E.F.G.

## 83. Short Time High Temperature Pasteurization—A Milk Dealer's View. W. D. DOTTERER, Bowman Dairy Co., Chicago, Ill. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year: 13, 332, 1939.

This method will accomplish essentially the same result as 30 minute holding pasteurization. Bacterial counts are perhaps slightly higher with short time pasteurization, although since a centrifugal clarifier has replaced the filter, plate counts have not been significantly different. A large number of phosphatase tests have never given any indication of improper pasteurization by the short time method as used in the plant under observation. Some of the advantages of short time pasteurization are less floor space required, less time for cleaning, lower initial cost, less time from start of pasteurization till milk is ready to bottle, less milk left in equipment to be over pasteurized in case of a shut down and less time between the end of the pasteurizing runs and the finish of bottling. The principal disadvantage is probably the narrower margin of safety between thermal death point of

pathogenic bacteria and the point at which the creaming of the milk is injured. Good controls eliminate this objection. E.F.G.

**84. Information Obtained by the Microscopic Examination of Raw Milk Not Shown by the Methylene Blue Test or the Standard Plate Count.** W. K. FOX AND G. J. TURNEY, Lansing Dept. of Health, AND C. S. BRYAN, Mich. Agr. Exp. Sta. Milk Dealer, 28: 12, 42-48, 1939.

Data are presented showing the comparison of the standard plate and the microscopic clump count on various classes of milk as determined by the methylene blue test. The authors conclude that the microscopic clump count and standard plate count compare very favorably when used on producer samples of raw milk to determine the bacterial content of such milk.

The microscopic method presents not only a method of counting, but also a means of determining the causes of poor quality in high count milk. The morphological types of bacteria present indicate the probable source of excess contamination in the system of milk production. This definite information can be obtained easily, quickly, and accurately through the microscope examination, thus facilitating more effective field work in a quality control program.

The amount of visible dirt in milk as shown by sediment tests has very little bearing upon the bacteria count of the milk. C.J.B.

**85. Milk Delivery Costs.** ANONYMOUS. Milk Dealer, 28: 12, 38, 62-68, 1939.

A study on milk distribution in Maine markets showed that delivery cost on routes where horses were used was about the same as that on routes of similar length where automobiles or motor trucks were operated. The daily delivery cost per route was \$1.30 for the 11 routes using one horse and \$1.36 for the 118 routes, less than 15 miles long, that used a motor vehicle. The volume delivered per route was slightly less on the routes using horses, resulting in a cost of \$1.57 per 100 quarts of retail milk equivalent, as compared with \$1.34 for routes using motor vehicles. The delivery cost per mile was similar for each group, averaging nearly 15 cents per mile with horses and 14 cents per mile with automobiles or motor trucks.

C.J.B.

**86. The Missing Factor in Chocolate Milk Sales.** A. P. PEYRAUD. Milk Dealer, 28: 12, 34, 72-73, 1939.

It is pointed out that the missing factor in chocolate milk sales is the habit of use and enjoyment of this product. To supply this missing factor the author suggests that in advertising chocolate milk the "milk" element

of the product should be so lightly touched upon as to be negligible, and the "chocolate" element should be handled with delicate restraint. The beverage element, the drink idea, however, must be loudly, widely, and strikingly proclaimed.

C.J.B.

**87. Refrigeration for the Milk Industry.** RALPH COPP, Pevely Dairy Co., St. Louis, Mo. *Milk Dealer*, 28: 12, 32-33, 85-88, 1939.

A complete discussion of refrigeration for the milk industry from farm to plant. Different refrigerants, operating costs, oil for compressors, as well as types of coolers and refrigeration plants, are discussed. The author stresses the importance of getting the proper equipment in the proper place.

C.J.B.

**88. Is There a Milk Monopoly.** THEODORE G. MONTAGUE, The Borden Co., N. Y. C. *Milk Dealer*, 28: 9, 94-97, June; 28: 10, 46-50, July; 28: 11, 72-75, August; 28: 12, 78-84, Sept., 1939.

A portion of a statement presented by Mr. Montague to the Temporary National Economic Committee, constituted pursuant to Joint Resolution of Congress. The author summarizes the points he has made as follows:

"The plain facts are that The Borden Co., handling less than five percent of the nation's milk supply, with its sales and profits decreasing and its competitors increasing in numbers, is not a monopoly. Moreover, there is no monopoly of distribution in the milk industry. High prices for milk result from monopolistic practices on the part of some government protected producer's associations and labor unions without forward looking leadership.

The plain facts are that the reduction in farm prices for milk in the early years of the depression was due to declining national market prices, and that retail prices were reduced as much as or more than farm prices—even more than the slight and temporary reduction in wages, the other big cost factor, warranted.

The plain facts are that the classified method of milk purchasing was established by the producers for the purpose of returning to themselves a higher price for milk sold as fluid milk. It was not designed to and does not now give our company an advantage in purchasing. Through abuses of this classified buying plan, however, farm cooperatives and governmental price-fixing have established artificially high prices for milk, thereby increasing production and discouraging consumption.

The plain facts are that consumers have a free choice as to whether they will purchase their milk from stores or have it delivered to their homes and, as a matter of fact, store sales have materially increased during recent years; and that the labor unions are the ones chiefly concerned over this

trend to store sales, because of the fact that deliveries to stores require fewer milk wagon drivers.

In my opinion conditions in the milk industry would be substantially improved if steps were taken to do the following:

First: Establish and maintain a sound relationship between the price paid the farmer for milk which is used for fluid consumption and the price paid for milk used in manufacturing. It is the latter price which establishes the fundamental value of all milk. The Borden Company believes in proper cooperative activities on the part of producers. It must be borne in mind, however, that the abuse of the power of collective bargaining by certain cooperatives, which are government protected\* monopolies, has resulted in arbitrarily high prices to a limited group of producers. These, in turn, have produced large surpluses to compete with the products of many thousands of farmers who have no opportunity to share in the high fluid milk markets. These practices have also resulted in high fluid milk prices to consumers.

Second: Reduce excessive wage costs. The Borden Company believes thoroughly in the right of labor to bargain collectively and in the payment of high wages in return for full measure of services. It should likewise be borne in mind, however, that the abuse of the power of collective bargaining by certain labor unions, which too are government protected monopolies, has also resulted in artificially high prices for fluid milk to the consumer.

If these two basic abuses by organized producers and organized labor are eliminated the following results may be expected as a matter of course:

A. The retail price of milk will find its natural lower level.

B. Employment will be stabilized throughout the industry and at good wages.

C. The farmer will receive a sound price for his milk for fluid use, and production and consumption will be more nearly in balance.

D. The burden of carrying the surplus will no longer be such an onerous one, and all producers and all distributors will be more nearly on an equal basis.

E. There will be no possible justification for governmental price fixing.

Even if for some social reason governmental price-fixing is to be continued in some form, it is imperative that there be a sound relationship between the price to farmers for fluid milk and the price of milk used in manufactured milk products. The present large differential cannot continue to exist.

For reasons founded in its own self-interest The Borden Company cannot forget that the consumer comes first. The very nature of the business, the comparative ease with which new competitors find a place in it through price cutting if profits are high, make low prices as necessary to the established dealers as they are desirable to consumers. Thus a distributor either

\* Government protected because of exemptions in the anti-trust laws.

stands or falls by his success or failure in keeping his price policies in line with competitive conditions.

The principle of governmental price control and the abuse of raw milk and labor costs by farm cooperative associations and labor unions fail to recognize these inescapable facts. As a business man attempting to sell more goods to more people, I would be concerned for the future of my company and its ability to operate in the public interest if I felt that this committee were to overlook these fundamental truths."

C.J.B.

### MISCELLANEOUS

- 89. Water Conditioning in Milk Plants.** G. A. RICHARDSON, University of California, Davis, Calif. Assoc. Bull., Intern. Assoc. Milk Dealers, 32nd year, No. 2, 54-58, 1939.

Six ways are listed by which water may be softened or the calcium and magnesium rendered unavailable for the formation of insoluble soaps. These are, distillation, heating raw water, lime treatment, lime-soda hot or cold, base exchange and addition of common alkalies. The complexity of boiler water treatment is pointed out. The modern method with condenser waters is to add a chemical to keep the calcium salts in solution rather than to precipitate them out.

Scale and milk stone are definitely attributed to water and alkaline detergents. In vats milk stone can be prevented by using soft water or zeolite softened water or by the use of one of the newer molecularly dehydrated phosphates which include sodium salts of phosphates referred to as pyro-phosphate, metaphosphate and tetra-phosphate. These form a complex with calcium or magnesium which does not form insoluble soaps and imparts improved rinsing properties. All three of the above have been used with success with alkalies in sufficient concentration to soften the water with some to spare. Each operator has a right to demand one of these chemicals as an ingredient of specific proprietary compounds because of their efficiency in preventing scale and milk stone.

E.F.G.

- 90. The Approach to and Results of 1939 Legislation.** W. A. WENTWORTH, Dairy Industry Committee, New York City. Assoc. Bull., Intern. Assoc. Milk Dealers, 32nd year, No. 3, 81-94, 1939.

Perhaps the outstanding feature of the past year was the fact that the U. S. Supreme Court sustained the constitutionality and legality of A.A.A. control of prices and pooling operations in the Boston and New York markets. Figures are given tending to show that the producer does not receive the added price which the consumer pays in market under price control. An increase in manufactured products in the New England and North Atlantic states where A.A.A. and the states have controlled milk prices and

in the South under cotton restriction is noted. The investigations of the Temporary National Economic Committee has brought out considerable testimony with reference to monopolistic activities in the milk industry. At the present time about one-fourth of the blue (surplus) stamps issued by the Federal Surplus Commodities Corporation is used for butter. Fluid milk is sold to relief families through a special class at a lower price instead of through blue stamps. Other legislation affecting the dairy industry is discussed, including the wage and hour law, food and drug act, anti-trust laws and reciprocal trade agreements. Current and prospective national and state legislation on numerous issues is touched upon.

E.F.G.

- 91. Sanitary Floors for Dairy and Food Plants.** C. A. SHILLINGLAW,  
J. F. HALE, AND J. M. SHARE. *Can. Dairy and Ice Cream J.*, 18:  
7, 30, 1939.

Suitable types of floors for bottling and food processing plants should have the following characteristics: (1) a continuous smooth surface, (2) imperviousness to water or other materials used in plant, (3) ready ease of cleaning, and (4) ability to withstand loads and traffic. Wood floors are inexpensive but because they do not withstand continuous wetting they are best adapted to storage processing spaces which are reasonably dry. The success of a concrete floor is intimately connected with the method of laying and particularly the finishing of the surface. The wear and imperviousness of brick and tile floors is dependent upon the type of material and particularly upon the type of bonding material. Asphalt, mastic or patented surfacings make highly impervious smooth surfaces. Pitch of the floor and size of trap are important for good drainage. Proper use of cleaning agents is important if long use of floors is expected. Elimination of "slipping" hazards is important in order to avoid injury.

O.F.G.

- 92. 1938—Another Legislative Year.** W. A. WENTWORTH, Secretary,  
Dairy Industry Committee, New York City. *Assoc. Bull., Intern.  
Assoc. Milk Dealers*, 31st year: 10, 243, March, 1939.

A brief survey is made of federal and state legislature and administrative activities during the past five years. It is stated that the Agricultural Adjustment Administration has probably produced the greatest effect upon the milk industry. The use of milk and cream in terms of milk in New York City decreased 169 million pounds from 1929 to 1937 while production in five states, largely supplying this market, increased 419 million pounds. Butter production increased 33 per cent while evaporated milk increased 46 per cent and skim milk powder increased 75 per cent. The extent of dairy products purchasing by the A.A.A. is noted and especially the inauguration of purchase of fresh milk. The number of markets operating under an order of A.A.A. decreased to 22. The maximum was 49 in Decem-

ber, 1934. The probable effects of the Federal Trade Commission milk investigation, Fair Labor Standards Act, Social Security Law, etc., are noted. It is noted that under reciprocal trade agreements imports of dairy products have increased and exports decreased. The present status of state and federal filled milk legislation is analyzed.

E.F.G.

- 93. Reducing Costs of Motor Vehicles.** GLEN W. JOHNSON, Bowman Dairy Co., Chicago, Ill. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year: 13, 328, 1939.

Inspection and service routine is specified for short and long haul trucks. Diesels are recommended for long hauls because of economy and ease of handling.

E.F.G.

- 94. Dairy Waste Elimination and Sewage Disposal.** H. A. TREBLER, Sealtest, Inc., Baltimore, Md. Assoc. Bull., Intern. Assoc. Milk Dealers, 31st year: 16, 5-12, 1939.

Five types of liquid wastes are described, each of which may need to be handled separately from the standpoint of economy. Nine methods of disposing of spoiled or excess dairy products are outlined. In the case of rinsings and drippings it is suggested that these be saved for manufacturing purposes whenever possible or at least utilized for animal feed. It is suggested that only as a last resort should a real waste disposal plant be constructed. Experimental work with tin can trickle filters indicates good efficiency. The author states that the activated sludge process gives good results at reasonable cost. The tin can trickler system is, however, cheaper to construct and to operate.

E.F.G.

- 95. Social Security.** J. S. SEIDMAN, N. Y. Chapter, Nat'l Assoc. of Cost Accountants. Milk Dealer, 28: 12, 94, 1939.

A brief discussion of social security taxes from the standpoint of bonus payments, expenses incurred by employees and charges for "shortages" and credits for "overages."

C.J.B.

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## ABSTRACTS OF LITERATURE

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National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

### BACTERIOLOGY

96. **The Decomposition of Citric Acid by *Betacoccus cremoris*.** G. VAN BEYNUM AND G. W. PETTE. State Agr. Exp. Sta., Hoorn, Netherlands. J. Dairy Res., 10: 250-266, 1939.

Analyses reveal that the fermentation products of *Betacoccus cremoris* in neutral milk are acetic acid and carbonic acid. In acidified milk or in mixed cultures of this bacterium and lactic acid streptococci the products are acetic acid, CO<sub>2</sub>, diacetyl, acetyl methyl carbinol and 2-3 butylene glycol. Diacetyl is only formed when an oxidation with atmospheric oxygen can take place. Carbinol is found in aerobic and in anaerobic cultures. It may be reduced to butylene glycol. All of these substances are formed from the citric acid of the milk. From 1 mol. of citric acid are formed: 2 mol. of CO<sub>2</sub>, 1-1.5 mol. of acetic acid, 0.5-0 mol. of C<sub>4</sub> compounds. A reciprocal relationship is shown to exist between the quantities of acetic acid and C<sub>4</sub> compounds. When the amount of acetic acid is high the C<sub>4</sub> compounds content is low. The higher the acidity of the medium in which the betacocci are cultivated the higher is the amount of C<sub>4</sub> compounds. The authors assume that pyruvic acid is an intermediate product and present formulae showing the decomposition of citric acid.

S.T.C.

97. **The Nutritional Requirements of the Lactic Acid Bacteria.** J. G. DAVIS, Nat. Inst. for Res. in Dairying, Univ. of Reading, Reading, England. J. Dairy Res., 10: 186-201, 1939.

Serum and blood were found not to have a marked effect on the growth of lactic acid bacteria in milk. Heated serum and, to a greater extent, heated blood usually stimulated growth. Unheated serum was found to inhibit *S. lactis*, when a small inoculum of washed cells was used. Under practical conditions no effect was noticed. The author concludes, therefore, that "it is unlikely that 'slow starter', is ever attributable to infiltration of serum or the constituents of serum."

S.T.C.

### BREEDING

98. **Biennial Reviews of the Progress of Dairy Science.** Section G, Genetics of Dairy Cattle. J. Dairy Res., 10: 370-393, 1939.

A review of recent work relative to the genetics of dairy cattle.

S.T.C.

### BUTTER

99. **Bacteriological Testing of Butter. New and Simplified Routine Methods.** G. M. MOIR AND R. R. RUSSELL. New Zealand Dept. of Agr., Wallaceville, Wellington. J. Dairy Res., 10: 310-325, 1939.

Microplate cultures are prepared to obtain counts of total bacteria, "heat resistance" bacteria, lypolytic organisms, yeasts and molds in butter. The method for counting colonies of lypolytic bacteria is based upon their ability to produce around each colony a halo of white solid fatty acids in place of the clear glistening fat globules.

S.T.C.

100. **Oxidation of the Fat of Butter During Cold Storage.** W. J. WILEY, Dairy Res. Inst., Palmerston North, New Zealand. *J. Dairy Res.*, 10: 300-309, 1939.

The oxidation of the fat of butter during cold storage was studied by measuring the fat-aldehyde value of the fat. Acidity, starter organisms, salt and low-pasteurization temperatures were found to favor oxidation. Neither diacetyl nor acetoin influenced the oxidation.

The author postulates the presence in ripened pasteurized cream and in unripened raw cream of a fat-oxidizing enzyme which is most active at low pH values, and high salt concentrations.

S.T.C.

101. **Observation on Epidemics of Moldy Salted Butter.** V. L. TURGASEN, Armour & Co., Chicago. *Nat. Butter and Cheese J.*, 30: 11, 44, 1939.

Seven epidemics are described and their causes are indicated. Sources of contamination in order of importance are unclean equipment, air-borne infection, insufficient pasteurization exposures of the cream, contaminated tubs, boxes and liners and contamination from such sources as water, salt and butter cultures.

W.V.P.

## CHEESE

102. **The Relation of Certain Lactic Acid Bacteria to Open Texture in Cheddar Cheese.** I. R. SHERWOOD, Dairy Res. Inst., Palmerston North, New Zealand. *J. Dairy Res.*, 10: 326-335, 1939.

The rate of evolution of carbon dioxide from cheese was measured by determining the ml. of N/10 barium hydroxide neutralized by the gas from 25 g. of cheese in two days at room temperature. "Open" cheese was shown to evolve carbon dioxide much more rapidly than "close" cheese. From open cheese lactobacilli or betacocci capable of producing carbon dioxide relatively rapidly were isolated. The addition of such organisms to cheese milk resulted in the development of slit openness in the cheese.

S.T.C.

103. **A Simple and Accurate Viscosimetric Form of Rennet Test.** C. W. KING AND E. M. MELVILLE, The West of Scotland Agr. College, Glasgow. *J. Dairy Res.*, 10: 340-354, 1939.

A viscosity type of rennet test is described. A mathematical treatment of the problems involved in the use of the method is given. S.T.C.

- 104. The Assessment of Curd Firmness Prior to Cutting.** F. M. V. COPPEN, Nat. Inst. for Res. in Dairying, Univ. of Reading, Reading, England. *J. Dairy Res.*, 10: 336-339, 1939.

Using the method devised by Scott Blair (*J. Dairy Res.*, 9: 347. 1938) the "firmness" of cheese curds immediately prior to cutting was determined and related to an expert's opinion. The smallest difference in modulus which could be correctly appreciated by the cheese maker was of the order of 8 per cent. Normal curds, regardless of type, were softer in the summer months than in the winter.

S.T.C.

- 105. The Making of Processed Cheese.** H. H. SOMMER AND H. L. TEMPLETON, Univ. of Wis., Madison. *Wisconsin Agr. Exp. Sta. Res. Bull.* 137, June, 1939.

Processing by selecting raw cheese, blending, grinding, heating and packaging gives uniformity of quality and a desirable package. Some patents restricting manufacture of process cheese have expired. Historical background of the industry and legal definitions are given. The processing procedure is described including details of selecting and blending of cheese to obtain desired flavor, body properties and composition; trimming and grinding to remove inedible portions and to prepare the cheese for effective heating; heating and stirring to obtain a homogenous and fluid mixture for packaging; use of emulsifiers; and packaging. Processing and packaging machines are described. Preparation of cheese spreads is given. Factors to consider in planning cheese processing operations are presented to indicate that approximately 100,000 lbs. of raw cheese is necessary to permit proper blending operations and successful control of quality when the output is 1,000 pounds per day and the average age of cheese is 3 to 4 months; that the minimum investment in equipment would be about \$1500 to \$2000; and that efficient large scale operations require considerable additional investment in labor-saving machinery. One table, 5 figures, 20 references and a list of 102 U. S. patents showing date of issue, patentee and patent title.

W.V.P.

## CHEMISTRY

- 106. The Rapid Determination of Peroxide Values for the Fat in Milk Powders.** J. A. B. SMITH, The Hannah Dairy Res. Inst., Kirkhill, Ayr. *J. Dairy Res.*, 10: 294-299, 1939.

A method which although yielding only about 90 per cent of the true value is considered of value by the author due to its rapidity is described.

Ten g. of milk powder are weighed into a 100 ml. graduated flask and 50 ml. glacial acetic acid added. The mixture is warmed to 35° C. for 5 minutes and shaken at intervals during that time. Chloroform is added from a burette until the 100 ml. mark is reached, and during this addition the mixture is well-shaken several times. The mixture is filtered rapidly through an ordinary filter funnel. An aliquot of the filtrate is measured out, one ml. of a saturated solution of potassium iodide added and the mixture shaken for one minute. It is then diluted with 100 ml. of water and titrated with standard sodium thiosulphate, 0.01 to 0.002 normal.

S.T.C.

107. **The Preparation of Phenyl Phosphoric Esters.** E. J. KING AND T. F. NICHOLSON, British Postgraduate Medical School, London. Biochem. J., 33: 1182, 1939.

In this paper are presented methods for conveniently preparing several phenyl phosphoric esters. The use of these in the phosphatase test of milk is not discussed but they may be conveniently adopted. The procedures described are for the preparation of barium phenyl phosphate, disodium phenyl phosphate, ortho-methylphenyl phosphate, para-bromophenyl phosphate, para-nitrophenyl phosphate, cyclo hexanol phosphate.

K.G.W.

108. **The Rates of Enzymic Hydrolysis of Phosphoric Esters.** E. J. KING AND G. E. DELORY, British Postgraduate Medical School, London. Biochem. J., 33: 1185, 1939.

The relative rates of hydrolysis of several phosphoric esters is presented. The rate of hydrolysis and the optimum pH for hydrolysis appear to increase with increasing acidity of the phosphoric ester.

K.G.W.

109. **Estimation of Lactic Acid in Biological Material by Oxidation with Ceric Sulfate.** J. J. GORDON AND J. H. QUASTEL, Biochemical Laboratory, Cardiff City Mental Hospital, Cardiff, Wales. Biochem. J., 33: 1332, 1939.

A method is presented for estimating lactic acid in biological materials using ceric sulfate. The acetaldehyde resulting from the oxidation of lactic acid is absorbed by sodium bisulphite and estimated iodometrically. The error in estimation of lactic acid does not exceed 5 per cent.

K.G.W.

110. **Determining Riboflavin. A Fluorometric and Biological Method.** G. C. SUPPLEE, R. C. BENDER, AND O. G. JENSEN, The Borden Co., Bainbridge, N. Y. Ind. Eng. Chem., Analyt. Ed., 11: 9, 495-498, 1939.

Methods for extracting and preparing riboflavin solutions from various concentrates, adsorbates, whey and miscellaneous products and for the fluorometric examination of the riboflavin solutions in "black light" are presented. A method for the biological determination of riboflavin is given and the results obtained by both methods are compared. The methods show a correlation greater than 90 per cent when applied to dry yeast and raw peanuts, 80 to 85 per cent when applied to alfalfa meal and liver and less than 50 per cent for soy bean and corn meals. The methods may be used interchangeably for certain types of riboflavin solutions and adsorbates. The fluorometric method gives check results within 10 per cent variation from independent determinations with different operators and within 3 to 5 per cent with experienced operators.

B.H.W.

**111. Determining Riboflavin in Dried Milk Products.** ROYAL A. SULLIVAN AND L. C. NORRIS, Cornell Univ., Ithaca, N. Y. Ind. Eng. Chem., Analyt. Ed., 11: 10, 535-540, 1939.

Riboflavin is extracted from dried milk products by refluxing with a dilute solution of acid in 75 per cent acetone. Unstable colored impurities are removed by the addition of hydrogen peroxide to the solvent. The resulting solution is neutralized, filtered and used for the determination of riboflavin with a photoelectric photometer. Ninety per cent of the color of riboflavin is removed by reduction with sodium hyposulfite. By observation of the light absorption before and after reduction the concentration of the solution is calculated to  $\pm 0.05$  microgram per l. The photometer is calibrated with pure synthetic riboflavin. The photometer wiring diagram, a transmission curve for the light filters used, and other pertinent data are presented.

B.H.W.

**CONCENTRATED AND DRY MILK; BY-PRODUCTS**

**112. Bacteriological Studies of Canned Milk Products.** AGNES A. NICHOLS, The Hannah Dairy Res. Inst., Kirkhill, Ayr. J. Dairy Res., 10: 231-249, 1939.

A large number of evaporated milk and canned cream samples from general sources and from 3 factories in Scotland were examined. One hundred per cent of the tins examined from general sources were sterile. The factory samples, especially those of canned cream, had a lower sterility percentage, although it was considered that this was due to special circumstances of operation, and that ordinarily a sterile pack should be obtained. An outbreak of "buttons" in sweetened condensed milk was attributed to three causative organisms, one a species of *Actinomyces*, the other two species of the genus *Penicillium*.

S.T.C.

113. **Bacteriological Studies of Spray-Dried Milk Powder.** AGNES A. NICHOLS, The Hannah Dairy Res. Inst., Kirkhill, Ayr. J. Dairy Res., 10: 202-230, 1939.

Data are presented on over 400 samples of spray-dried milk powder secured from eight factories operating in England and Scotland. Plate counts varied from 1400 to 149,000,000 per gram. About 10 per cent of the samples tested in one ml. quantities gave positive presumptive coliform tests. The desirability of establishing grades for spray-dried milk powder is discussed.

S.T.C.

### DISEASE

114. **Biennial Reviews of the Progress of Dairy Science.** Section F, Milk Borne Diseases. J. Dairy Res., 10: 355-369, 1939.

A review of recent work relative to milk borne diseases.

S.T.C.

115. **The Casein Number for Diagnosis of Mastitis. II. The Effect of Advanced Lactation and of Storage and Preservation of Milk Sample.** S. G. ROWLAND AND M. ZEIN-EL-DINE, Univ. of Reading, Reading, England. J. Dairy Res., 10: 267-271, 1939.

The casein number (percentage of casein nitrogen in total nitrogen of the milk) method of mastitis diagnosis was found not applicable to cows in advanced lactation.

Storage of milk samples for more than a few hours at 16-18° C. resulted in a decrease in the casein number. Storage at 0-2° C., and at 16-18° C. with the addition of 0.2, 0.05, and 0.01 g. potassium dichromate, formalin or mercuric chloride respectively per 100 ml. of milk, prevented significant reduction of the casein number for 24-48 hours.

S.T.C.

### FEEDS AND FEEDING

116. **The Nutritive Value of Proteins for Milk Production. V. The Effect of High Temperature and of Season on the Nutritive Value of Grass Proteins, the Supplementary Effect of the Maintenance Ration on the Production Ration, and the Effect of Feeding a High-Protein Ration.** S. MORRIS AND S. C. RAY, The Hannah Dairy Res. Inst., Kirkhill, Ayr. J. Dairy Res., 10: 165-185, 1939.

As measured by milk production the nutritive value of artificially dried spring grass was unaffected by the temperature of drying even to the extent of scorching the grass. Dried spring grass was found to have a higher and later autumn grass a lower biological value than early autumn grass. Linseed cake was found to have a very low biological value. A supplementary

effect of the maintenance on the production ration was noted when hay was substituted for straw in the maintenance ration together with a production protein intake of poor biological value. With an excess of protein in the ration most of the excess nitrogen was secreted in the urine, while both the milk yield and the biological value of the food protein fell considerably. The authors suggest that together with an adequate amount of lysine in the protein ingested, a certain mixture of amino-acids is essential before maximum milk-yield can be obtained.

S.T.C.

## FOOD VALUE OF DAIRY PRODUCTS

117. **The Effect of Commercial Drying and Evaporation on the Nutritive Properties of Milk.** K. M. HENRY, J. HOUSTON, S. K. KON, AND L. W. OSBORNE, Nat. Inst. for Res. in Dairying, Univ. of Reading, Reading, England. *J. Dairy Res.*, 10: 272-293, 1939.

Spray-dried (Kestner process), roller-dried and evaporated milks were prepared commercially from one batch of raw milk. The biological value of the proteins (nitrogen), vitamin B, and the growth-promoting properties of the milks were measured by tests on rats. Vitamin A and carotene, riboflavin and vitamin C were measured by physical and chemical methods.

The proteins (nitrogen) of evaporated milk were found to be significantly less digestible (true digestibility (91)) than those of spray-dried milk (true digestibility (94)). The difference between either of those and roller-dried milk (true digestibility (93)) was not statistically significant. The vitamin B content of evaporated milk (9 I. U./100 ml. of reconstituted milk) was only half that of spray-dried (18 I. U./100 ml.) and of roller-dried (19 I. U./100 ml.).

Reconstituted spray-dried, roller-dried and evaporated milks were fed *ad libitum*, supplemented with mineral, as the sole diet to groups of littermate male rats. Based on gains in weight per 100 ml. milk intake the dried milks were found to be significantly superior to the evaporated milk.

Processing the milk caused no loss in vitamin A and carotene. Moreover, they were not decreased after a year's storage of the processed milks. There was no difference in the riboflavin content of the processed milks and the factor was not affected by 16 months' storage.

Spray-drying caused a 20 per cent loss, roller-drying and evaporation a 30 per cent loss in the original vitamin C content of the milk. S.T.C.

118. **Studies on Digestibility of Proteins in Vitro. VII. Liberation of Cystine on Tryptic Digestion of Casein with Observations on the Instability of Cystine Toward Alkali.** D. BREESE JONES AND CHARLES E. F. GERSDORFF, Protein and Nutrition Res. Div., Bureau of Chemistry and Soils, U. S. Dept. of Agr., Washington, D. C. *J. Biol. Chem.*, 129: 207, 1939.

This paper is a presentation of data on the liberation of cystine from casein at various reactions and with tryptic digestion.

Cystine is readily liberated from casein by tryptic digestion at pH 8-9. A large part of the casein cystine, however, is destroyed during the digestion by the alkalinity of the digest. When the digestions are conducted at slightly acid reactions, cystine was quite readily and completely liberated, although at a slower rate than when the digests were alkaline. There was no destruction of cystine. About 40 per cent of the casein cystine was liberated in 24 hours at pH 6.6-6.8. At pH 6.8 nearly all the cystine was liberated in 120 hours, while 168 and 216 hours were required when the digestions were conducted at pH 6.6 and 6.2, respectively. K.G.W.

- 119. The Effect of Orange Juice on Calcium Assimilation.** CAROLINE S. LANFORD, Dept. of Chemistry, Columbia Univ., New York City. *J. Biol. Chem.*, 130: 87, 1939.

Young growing rats were fed moderate quantities of orange juice in addition to a basal wheat and milk mixture. The amount of juice given daily (5 cc.) corresponded on the average to about 15 cc. per 100 Kilo calories of diet consumed.

The proportion of the calcium of the diet which was stored in the body under these conditions was about 8 per cent greater than the proportion stored by control animals on the basal diet alone. This improved assimilation of the dietary calcium was noted in every instance and was of unquestionable significance by the usual statistical criteria. K.G.W.

## ICE CREAM

- 120. Formulas for Ice Cream with Reduced Carbohydrate Content.** W. J. CORBETT AND P. H. TRACY. Ill. Agr. Exp. Sta. Cir. 498, Nov., 1939.

Commercial ice cream ordinarily has a carbohydrate content of about 20 per cent and is unsuited for diabetic patients.

Three formulas for ice cream mix with low carbohydrate content are suggested. Directions are given for making the low-lactose milk which is used in two of the formulas.

Caloric values per pint of these ice creams drawn at 80 per cent overrun are shown.

A formula for home use is included.

O.R.O.

- 121. Plant Layout.** H. J. BROWN, Goldenrod Ice Cream Co., Chicago, Ill. *Ice Cream Field*, 34: 4, 21, 26, Oct., 1939.

The author points out the advisability of planning the plant layout so that each operation can follow in proper routine with the elimination of

unnecessary handling of the products. He favors the one floor plan for manufacturing operations, and claims that continuous ice cream freezers are more suited for economical operation than batch freezers.

In general he states that all ice cream plants are similar, but that the peculiarities of each must be considered in order to be assured of efficient production.

W.C.C.

**122. Raw Materials for Ice Cream.** J. HOFFMAN ERB, Dairy Dépt., Ohio State Univ. *Ice Cream Field*, 34: 4, 22, 23, Oct., 1939.

The author discusses the necessity of controlling the quality of raw materials used in the manufacture of ice cream. He points out that oxidized flavor is one of the most common defects in ice cream and states that from a practical point of view the elimination of copper from the mix is the most successful means of preventing it.

According to the author many plants now make Eh determinations on cream and in one instance cream is never frozen or stored for subsequent use if it has an Eh of 0.350.

W.C.C.

**123. New Processes of Mix Manufacture.** E. C. SCOTT, Research Laboratory, Swift and Company, Chicago. *Ice Cream Field*, 34: 4, 24-25, Oct., 1939.

The production of high quality ice cream at the lowest cost is the aim of the ice cream industry the author points out. It is stated that it is possible to produce high quality ice cream using either high or low percentages of serum solids, provided the proper balance is maintained between serum solids, stabilizers and other solids that will permit the ice cream to be drawn from the freezer in a stiff, fairly dry condition. When sweet cream is not available the tendency is towards the use of frozen cream in place of butter, although there is some indication that the findings of Leighton and Levitan (*Ind. Eng. Chem.*, June, 1939) may tend to increase the use of high quality unsalted butter by first homogenizing the butter and skim milk to form cream.

It is claimed that soft ice cream as drawn from the freezer can often be traced directly to the use of stabilizers with a high sodium salt content.

The tendency to use higher sugar contents in ice cream has necessitated higher pasteurization temperatures. It is claimed that prolonged heating at temperatures from 150° F. to 180° F. results in the precipitation of calcium salts and this in turn impairs the body of the finished ice cream. The author states that pasteurizing and holding vats with stuffing boxes and horizontal type coils are becoming obsolete, due to the increased emphasis on sanitation.

It is stated that making ice cream mixes in the vacuum pan is not gaining in popularity. In the case of mixes made from mediocre quality prod-

uets, it may have a slight advantage whereas otherwise it tends to make a flat-tasting mix.

Direct expansion cabinet coolers are rapidly replacing brine and sweet water coolers and immediate cooling to 34° F. to 40° F. is the general practice. Homogenizers of current design are either of the two stage type or else a single stage type with auxiliary features to give results that approximate double stage homogenization. The newer homogenizers generally accomplish satisfactory homogenization at lower pressures than was formerly required. According to the author the presence of fat globules larger than 3½ microns in diameter indicates incomplete homogenization. W.C.C.

- 124. Modern Freezing Methods.** A. W. FARRALL, Creamery Package Manufacturing Co. Ice Cream Field, 34: 4, 27, 28, 29, 44, Oct., 1939.

It is pointed out that the three principal requirements of freezing ice cream are:

1. The ice cream mix must be cooled rapidly to a temperature of from 22° F. to 24° F.
2. The proper amount of air must be incorporated uniformly in a fine state of division.
3. The freezer or its attachments must be capable of uniformly mixing fruits, nuts, candy and the like in the ice cream.

The author states that over-neutralization of the mix or the use of ingredients which contain a high percentage of sodium caseinate cause the ice cream to become sticky, thus increasing the power consumption of the freezer. Low whipping mixes cause difficulty in obtaining overrun with batch freezers and with continuous freezers may cause air slugs in the ice cream. Ordinarily as the temperature is lowered from 26° F. to 24° F. the ability to incorporate air is increased, whereas lowering the temperature below 23° F. air is whipped in with more difficulty and usually at 22° F. it is necessary to maintain a fairly high air pressure in order to obtain the desired overrun. This condition is made possible in the continuous freezers on the market.

The advantages of rapid freezing as a means of improving ice cream texture are mentioned and instructions for sharpening freezer blades properly are given and illustrated diagrammatically.

In modern freezing both batch and continuous freezers are in use. Efficient plant layout and arrangement of schedules contribute to economical plant operation. W.C.C.

- 125. Hardening of Ice Cream.** P. H. TRACY, Dept. of Dairy Husbandry, Univ. of Ill., Ice Cream Field, 34: 4, 30, 31, 44, Oct., 1939.

It is claimed that from 10 to 12 inches of good insulation is advisable in the walls and ceiling, and at least 8 inches on the floors of hardening rooms.

Ordinarily the hardening room should be large enough to hold about three times the amount of the peak day's run (roughly one per cent of the annual production). In the case of novelties and package goods, a larger space per gallon is necessary.

Three types of hardening rooms are mentioned: 1. Hardening rooms with shelf and ceiling coil arrangement, 2. Hardening rooms with bunker system, 3. Hardening rooms with the blower type of refrigeration unit. The merits of each type are mentioned.

The advantages of fast hardening are indicated and it is pointed out that air circulation in the hardening room markedly contributes to this. A brief review is given of a paper by Tracy and McGown (J. DAIRY SC. 17: 47-60).

W.C.C.

**126. Shrinkage of Ice Cream.** W. C. COLE, Dairy Industry Div., Univ. of Calif. Ice Cream Field, 34: 4, 32-34, Oct., 1939.

The influence of changes in external pressure is considered by the author as contributing to the problem of ice cream shrinkage.

Products manufactured at sea level and shipped to altitudes five or six thousand feet above may increase considerably in volume, whereas those manufactured in higher altitudes and shipped to sea level often show shrinkage.

Experiments were carried out by placing samples of ice cream in a vacuum pan and observing changes in volume of the ice cream accompanying changes in pressure. The results are illustrated in tabular form and by means of photographs.

The author concludes as a result of this study that:

1. Variations in external pressure may cause marked changes in the volume of ice cream.

2. When such pressures are reduced, the volume of ice cream increases. When the pressure is increased again to the original value, ordinarily the ice cream does not return to its original volume; hence we say that it has shrunk. This shrinkage is the result of loss of air.

3. Some of the shrinkage in commercial ice cream particularly where marked changes in altitude are involved, may be explained on the basis of the principles just indicated.

4. When ice cream is subjected to these changes in pressure its hardness, which depends primarily upon temperature, is an important factor determining the extent to which shrinkage occurs. Other things being equal, the harder the ice cream the less will be its change in volume as a result of variations in pressure.

W.C.C.

**127. Flavoring Characteristics of Individual Cocoa Varieties.** P. S. LUCAS AND I. A. GOULD, Michigan State College. Ice Cream Field, 34: 5, 28, 29, 34, Nov., 1939.

The authors claim that the individual flavoring characteristics of the liquor from particular types of cocoa beans are unknown because of the custom manufacturers have of blending or combining various beans before or during their processing. They point out further that commercial chocolates and cocoas in many cases are modified by the addition during milling of vanillin, essential oil of almonds, cinnamon, cassia, ginger, tolu balsam and other materials. They selected seven representative varieties of cocoa beans on the New York markets. The average quality, production and price of these varieties are given in the following table. In addition they are described briefly.

*Average quality. Production and price of cocoas*

Variety	Quality Rating	Production	Price N. Y. Market May 4, 1939 (cents)
1. F. F. Accra	Sixth	First	4.5
2. Arriba	Third	Fifth	8.0
3. Bahia	Fifth	Second	4.5
4. Puerto Cabello	First	Sixth	12.0-18.0
5. Caracas	Second	Seventh	10.0
6. Sanchez	Seventh	Third	4.1
7. Trinidad	Fourth	Fourth	7.5

Ice creams of the same composition except for the flavor used were prepared as a means of arriving at public preference for chocolate flavors peculiar to particular varieties. The authors report that their tests indicate the public preference for chocolate varieties in ice cream is for the stronger varieties of beans having chocolate flavor of the bitter type. They state that the commercial practice of blending several varieties of cocoa beans to avoid seasonal changes in flavor and to provide color and other desirable effects is justified.

W.C.C.

**128. Public Health Standards for 1940.** DAVID LEVOWITZ, New Jersey Dairy Laboratories. *Ice Cream Field*, 34: 4, 45-46, Oct., 1939.

The purpose of the article is to indicate not only what changes are to be expected in ice cream control during the next year, but also how the ice cream manufacturers may prepare for them. The author considered the following to be important:

*Ingredients*

- a. *Dairy ingredients*—Standards applying to fresh fluid products for sale as such are to be applied to these products when used in the manufacture of ice cream.

b. *Non-dairy ingredients*—They must be of approved type, pure, and stored in such a manner that they are not contaminated, and given such treatment as is necessary prior to their addition to the mix.

#### *Processing Equipment and Practice*

a. *Pasteurization*—Heating to 155° F. (or higher) for 30 minutes by the “long hold” process; or where approved, 180° F. (or higher) for 16 seconds by high temperature-short hold process.

b. *Homogenization*—May be performed by any approved emulsifying mechanism. Only equipment which can be easily taken apart for cleaning and sterilization should be approved.

c. *Cooling, freezing, and packing*—The mix must be cooled to below 50° F., immediately after the conclusion of its (pasteurization or) homogenization. Molds for the freezing of bars, etc., are to be constructed without lead on surfaces coming into contact with the food products.

d. *Packages*—Metal cans used for bulk shipments must be made without lead on surfaces coming into contact with the food product. Metal containers must be cleaned and sterilized prior to use. Paper containers must be made of stock of the quality approved for the manufacture of milk containers. Assembly for containers must be done in an approved manner.

#### *Finished Product's Sanitary Standards*

a. *Samples*—Ice cream must be sampled with aseptic precaution from a full plant-package container, to determine the condition of the ice cream as it left the manufacturing plant.

b. *Phosphatase test*—Indophenol in phosphatase tests beyond that obligatory through controls is definite evidence of inefficient pasteurization.

c. *Total count*—A.P.H.A. tryptone-dextrose dairy nutrient agar is to be employed for the total colony count which must be well below 100,000.

d. *Direct microscopic count and types*—The standards employed for other dairy products will apply also to an area's ice cream.

e. *Coliform group significance*—Presence of this group indicates contamination. Confirmatory and corroboratory tests should be run to avoid the reporting of false positives.

The author discusses each of the factors listed above and points out further that considerable attention is now being given to ice cream dispensing sanitation. Those anticipating the installation of various types of equipment should anticipate future sanitation requirements. W.C.C.

**129. Let's Face Facts on Quick Frozen Foods.** C. Q. SHERMAN, C. Q. Sherman Corp. Ice Cream Field, 34: 6, 12, 13, 51, Dec., 1939.

The author claims that ice cream manufacturers should be interested in frozen foods mainly because frozen food sales are greatest when ice cream

sales decrease. He claims the best type of outlet for frozen foods is the grocer or meat dealer catering to the average housewife. To begin with, he considers that only staple articles should be sold such as peas, beans, spinach, asparagus, strawberries, raspberries, etc.

It is pointed out that frozen foods must be displayed to be sold. The author warns against financing the dealers but recommends carrying insurance against mechanical breakdowns resulting in defrosting and spoilage.

W.C.C.

**130. Determining Sanitary Quality by Laboratory Tests.** F. E. NELSON, W. J. CAULFIELD AND W. H. MARTIN, Kansas Agr. Exp. Sta. Ice Cream Field, 31: 6, 14, 15, 16, 28, Dec., 1939.

The following factors, it is stated, are commonly used to measure ice cream quality: (1) taste, (2) physical characteristics such as body and texture, (3) quantitative factors such as amount of overrun, butterfat and solids content, and (4) the sanitary quality of the product. The sanitary quality is much more difficult to evaluate and control than the other factors listed, according to the authors.

They consider the number and types of bacteria present in ice cream to be due to (1) bacteria which enter the product at any point from the udder of the cow to the mouth of the consumer, (2) opportunities for contaminating organisms to grow once they have gained entrance, and (3) the destruction of bacteria by such means as pasteurization.

The authors report results of laboratory tests on 315 ice cream samples in which the latter were subjected to standard plate count for bacteria, the direct microscopic count, the Escherichia-Aerobacter or Coliform bacteria test, and the phosphatase test. The results obtained are presented in tabular form and their importance discussed. It is stated that the outstanding lesson to be obtained from the results is that a definite reason can be found for almost all plate counts above 50,000 per ml. and for many of the counts above 25,000 per ml. by using only these four simple laboratory tests.

In conclusion the authors state that there is no substitute for continuous care and vigilance on the farm, in the plant, and in the retail establishment but that a well run laboratory can do much to prevent irregularities in sanitary quality.

W.C.C.

## MILK

**131. The Phosphatase Test for Control of Efficiency of Pasteurization.**

H. D. KAY, R. ASCIJAFFENBURG AND F. K. NEAVE, Imperial Bureau of Dairy Science, Shinfield, near Reading, England. Technical Communication No. 1, Oct., 1939.

The article is a critical review of the phosphatase test, covering com-

pletely the development, modifications and application of the test to milk and various milk products. It summarizes the results obtained by various investigators pointing out the advantages and limitations of the test, the sensitivity, and the effect of various factors such as storage, preservatives, bacterial contamination, stage of lactation, and mastitis on the reliability of the test.

The conclusions are that the test for milk is very sensitive, accurate, and effective in controlling the efficiency of pasteurization by the "holder" process. The test in its appropriate modification is sufficiently sensitive to detect a drop in temperature of 1.0 to 1.5° F. if pasteurizing temperature is 143° F. for 30 minutes. If 145° F. for 30 minutes a drop of 1.5° F. can be detected.

A reduction of 10 minutes in holding time can be detected but not 5 minutes with certainty.

The presence of 0.2 to 0.25 per cent of raw milk in pasteurized product can be detected and in many instances even smaller amounts.

Regarding the application of the phosphatase test to the high temperature short time holding process, it is concluded that, "the claim that the phosphatase test remains sensitive under H.T.S.T. conditions is well founded, but further experimental evidence is required to settle a number of details."

It is further concluded that the accuracy of the test when applied to products other than milk is still disputed, and further investigation is required with reference to amount of sample to be used for testing, limiting color standards, etc.

It is confidently expected that further research will achieve tests for milk products which will be as accurate and reliable for controlling the pasteurizing process as that already achieved for milk.

The bulletin also has an appendix giving precautions and detailed instructions for performing the phosphatase test. Technique for the following test and modifications are given.

- A. Kay-Graham Test A and Test B.
- B. Neave's Modification.
- C. Gilcreas and Davis Modification.
- D. Scharer's Modification.
  - 1. Laboratory Test
  - 2. Field Test
- E. Leahy's Modification.
- F. Aschaffenburg and Neave's Modification.

The price of the bulletin is two shillings.

L.H.B.

**132. Accuracy of Methods of Sampling Milk Deliveries at Milk Plants.**  
P. H. TRACY AND S. L. TUCKEY. Ill. Agr. Exp. Sta. Bull. 459,  
47-84, 1939.

A study of the accuracy of the methods used to sample the milk delivered at each of four dairies.

From the data secured from these dairies the following conclusions are drawn:

1. Inaccurate tests may result from improper mixing of the milk when dumped into the weigh tanks.
2. To determine the accuracy of sampling from weigh tanks, samples from each tank without previous stirring should be checked against samples taken when the milk has been thoroughly stirred.
3. Tests on composite samples properly taken and kept will give an accurate measurement of the fat content of the milk.
4. Periodic sampling would not be satisfactory on a market where variations in daily tests are wide.
5. Variation in daily tests on milk from the same patron was sufficiently great to indicate mechanical manipulation of the fat content.
6. The tendency for plant composite samples to test less than laboratory composite samples is thought to be due to variations from the accepted practice in the care of the samples.
7. Composite samples need not be taken in aliquot portions to give results that will be sufficiently accurate for practical purposes. O.R.O.

**133. Development and Present Status of the Big Milk Bottle.** ANONYMOUS. *Milk Plant Monthly*, 28: 9, 24, 1939.

The demand for lower priced milk brought the large container into the milk distribution field. The development of the present most favored two-quart or half-gallon bottle from the gallon jug is discussed, giving consumer and distributor opinions. The gallon container with a handle appealed to the consumer because of its convenience in carrying, but distributor opinion was that the consumer who had not used the one gallon container would prefer the two-quart bottle at the same price per unit of milk. The majority of the distributors questioned favored the two-quart bottle over the gallon container.

G.M.T.

**134. The Relation of Nutrition of the Cow to Development of Oxidized Flavor in Milk.** J. L. HENDERSON, Div. Dairy Ind., Univ. of Calif., Davis, Calif. *Milk Plant Monthly*, 28: 12, 26, 1939.

Eighteen papers on oxidized and rancid flavors in milk are reviewed showing that rations high in carotene result in a milk less susceptible to metal-induced oxidizer flavor and to the development of rancidity. For this purpose carotene concentrate, carrots and various green feeds have been used successfully. Maintaining a satisfactory carotene content in the milk throughout the year seems now to be the part of proper dairy herd management, thus assuring not only a more stable milk but aids in maintaining a uniform vitamin A potency as well.

G.M.T.

135. **A Comparison of the Imperviousness of Commonly Used Paper Milk Containers.** R. B. STOLTZ AND T. V. ARMSTRONG, Dept. of Dairy Techn., Ohio State Univ., Columbus, Ohio. Milk Plant Monthly, 28: 12, 54, 1939.

Five different makes of quart paper bottles two of square and three of cone construction were studied for absorption of liquids and penetrability of dyes. None of the bottles studied was found to be impervious. Two of the three bottles paraffined in the factory, cone shaped, offered the best protection against the entrance of moisture to the fibre. There was a tendency toward greater absorption of moisture at the higher temperatures. A 40 per cent cream seemed less penetrable than homogenized milk or skim milk. The weight increase per bottle ranged from 0.11 grams to 1.55 grams.

G.M.T.

136. **Physico-Chemical Principles Involved in Controlling Cream Body.** H. H. SOMMER, Univ. of Wis., Madison, Wis. The Assoc. Bull., Intern. Assoc. Milk Dealers, 32nd year: 113-117, Dec., 1939.

Attention is drawn to the complex nature of milk and an extensive catalogue made of conditions promoting or decreasing viscosity. The structure of cream is conceived to be due to clusters of fat globules in contact, one with another. The difference in effect when the adsorbed film is acquired by solid as compared with liquid fat globules is emphasized. A hypothesis is given to explain the functioning of the cooling-warming-cooling method of increasing viscosity from a physico-chemical viewpoint.

E.F.G.

137. **Studies on the Structural Aspects of Paraffined Paper Containers.** C. S. MUDGE, D. C. FOORD, J. L. HENDERSON. The Assoc. Bull., Intern. Assoc. Milk Dealers, 32nd year: 107-112, Dec., 1939.

Completeness of paraffining of paper milk containers was measured by extent of staining of the board when an iodine solution was allowed to remain in the container for 5 minutes. Containers stored at various humidities for 2 to 3 weeks were examined bacteriologically, but no evidence was obtained that bacteria work to the surface of the paraffin layer. Sediment tests made on paraffin films dissolved off with gasoline showed negligible sediment. Unbleached paper stocks resulted in higher ascorbic acid values and less impairment in flavor when the milk was exposed to direct sunlight for two hours than when bleached paper was used. An inner layer of heavily dyed paper is suggested as a possible improvement.

E.F.G.

138. **Microscopic Examination of Pasteurized Milk.** H. MACY, Univ. of Minn., St. Paul, Minn. The Assoc. Bull., Intern. Assoc. Milk Dealers, 32nd year: No. 4: 97-106, Dec., 1939.

The standard plate method and the Breed microscopic method were used on "street" and "plant" samples of pasteurized milk and on milk pasteurized in the laboratory. Dividing 275 "street" samples into six groups in the low count group the ratio of the plate to microscopic count was 1:260 while in the high count group the ratio was 1:25. The plant and individual patron samples followed somewhat the same general pattern. Laboratory pasteurized samples commonly decreased in microscopic count at least 50 per cent following pasteurization. Thermophyles were revealed by a microscopic count which were not fully accounted for by the regular plating method for the determination of thermophyles. The results suggest that microscopic examination of milk may yield some helpful information. The types of bacteria in the raw milk will have a good deal to do with the results obtained by microscopic examination of the pasteurized milk.

E.F.G.

### MISCELLANEOUS

139. **The Distributor Is the Chief Factor in the Successful and Adequate Consumption of Dairy Products.** Mrs. W. E. FRIBLEY, Chicago Housewives League, Milk Plant Monthly, 28: 6, 52, 1939.

Price alone does not seem to be the chief factor in the purchase and utilization of more milk by the average housewife, but rather indifference to dairy products. The consumer must be interested in dairy products, not by the milkman whom no housewife considers the proper person to educate her to use more dairy products, but through effective advertising, placards, pictures, radio programs, menus and luncheon parties. Many mothers know the children should receive a quart of milk a day but grow weary of trying to induce them to drink it, since the children themselves have not been interested. The suggestion is made for the need of adequate quick cooking dairy dishes, for more information concerning the uses of milk, and for protected doorstep delivery as aids in increasing consumption of dairy products in the home.

G.M.T.

140. **Looking into This Washing Powder Problem.** A. H. BAYER, Nat. Dairy Products Corp., Schenectady, N. Y. Nat. Butter and Cheese J., 30: 11, 52, 1939.

For good cleaning and prolonging of life of equipment study each cleaning job, do not use washing powder in excess, see that equipment is properly washed, be sure equipment is left dry after cleaning and sterilizing, get a reliable vendor to survey your requirements and use his service.

W.V.P.

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## ABSTRACTS OF LITERATURE

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## MILK AND MILK PRODUCTS

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Baltzer, A. C.	Erb, J. H.	Knight, D.	Richardson, G. A.
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Bartlett, J. W.	Fabian, F. W.	LaMaster, J. P.	Schultz, E. N.
Becker, R. B.	Frayer, J. M.	Leighton, A.	Sommer, H. H.
Bendixen, H. A.	Frazier, W. C.	Lindquist, H. G.	Stark, C. N.
Bennett, F. W.	Fuller, J. M.	Lucas, F. S.	Swope, W. D.
Bird, E. W.	Garrett, O. F.	Lush, J. L.	Tarasuk, N. P.
Brueckner, H. J.	Gelpi, A. J.	Mack, M. J.	Theophilus, D. R.
Burgwald, L. H.	Golding, N. S.	Macy, H.	Thomsen, L. C.
Burke, A. D.	Goss, E. F.	Mann, A. L.	Totman, C. C.
Burri, R.	Greenbank, G. R.	Marquardt, J. C.	Trout, G. M.
Bushnell, L. D.	Gullickson, T. W.	Martin, W. H.	Tuckey, S. L.
Cannon, C. Y.	Guthrie, E. S.	Mead, S. W.	Webb, B. H.
Carpenter, D. C.	Hansen, Arne	Moore, L. A.	Weckel, K. G.
Cave, H. W.	Herrington, B. L.	Morris, A. J.	White, G. C.
Clevenger, W. L.	Herzer, F. H.	Mueller, W. S.	Wilbur, J. W.
Coie, W. C.	Holdaway, G. W.	Nelson, D. H.	Wilster, G.
Copeland, L.	Horrall, E. E.	Nelson, J. A.	Wylie, C. E.
Coulter, S. T.	Huffman, C. F.	Overman, O. R.	Yale, M. W.

### JOURNALS

American Butter Review	Journal of Genetics
American Milk Review	Journal of Infectious Diseases
American Journal of Diseases of Children	Journal of London Chemical Society
American Journal of Physiology	Journal of Milk Technology
American Journal of Public Health	Journal of Nutrition
Archives of Pediatrics	Journal of Pathology and Bacteriology
Biochemical Journal	Journal of Physical Chemistry
Biochemische Zeitschrift	Journal of Physiology
Canadian Dairy and Ice Cream Journal	Kaseindustrie
Canadian Public Health Journal	Kolloid-Zeitschrift
Certified Milk	Lancet
Cornell Veterinarian	Le Lait
Dairy Industries	Milchwirtschaftliche Forschungen
Dairy World	Milchwirtschaftliche Zeitung
Deutsche Molkerei Zeitung	Milk Dealer
Endocrinology	Milk Industry
Food Industries	Milk Plant Monthly
Food Manufacture	Molkerei Zeitung
Food Research	National Butter and Cheese Journal
Guernsey Breeders Journal	Pacific Dairy Review
Ice and Refrigeration	Proceedings of Society of Animal Production
Ice Cream Field	Proceedings of Society of Experimental Biology and Medicine
Ice Cream Industry	Refrigerating Engineering
Ice Cream Review	Scientific Agriculture
Ice Cream Trade Journal	Tierernährung
Industrial and Engineering Chemistry	Tiersucher
Jersey Bulletin	Trudy Vologodskogo Molochnogo Institut
Journal of Agricultural Research	Zeitschrift für Infektionskrankheiten Parasitäre Krankheiten und Hygiene der Haustiere
Journal of Agricultural Science	Zeitschrift für Physikalische Chemie, Abt. A und B
Journal of American Chemical Society	Zeitschrift für Untersuchung der Lebensmittel
Journal of American Veterinary Medicine Association	Zeitschrift für Züchtung, Reihe B, Tiersuchung und Zuchtbioologie
Journal of Bacteriology	Zentralblatt für Bacteriologie
Journal of Biological Chemistry	Züchtungskunde
Journal of Dairy Research	
Journal of Dairy Science	
Journal of Experimental Medicine	
Journal of General Physiology	
Journal of Heredity	

### SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland	Prussian Dairy Research Institute, Kiel, Germany
International Association of Ice Cream Manufacturers	State Agricultural Colleges and Experiment Stations
International Association of Milk Dealers	The Royal Technical College, Copenhagen, Denmark
National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

### BOOK REVIEWS

- 141. Food Control: Its Public Health Aspects: A Manual for Regulatory Officers, Food Technologists and Students of the Food Industry.** JAMES HOUSTON SHRADER. Price \$4.00, 513 pages. Published by J. Wiley & Sons, Inc., New York, 1939.

This book is a general text, giving a broad comprehensive view on why food control is necessary, what methods are used commercially in such control and how these control measures are applied.

Since the handling of milk is of major interest as a food control subject, almost half of the book is upon milk and milk products. The remaining parts of the book deal with meats, eggs, fish, canned foods and other products.

The author gives good evidence for the proper pasteurization of milk, ice cream mixes, cream for butter making and milk used in making the various types of cheese. The effectiveness of pasteurization was brought out in the following manner. Based on the total number of officially reported milk-borne epidemics found in the United States for the years 1923-1935 and assuming that 2 per cent of the fluid milk consumed is raw certified, 49 per cent pasteurized and 49 per cent is ordinary raw milk, it was calculated that for every outbreak due to pasteurized milk there were 2.5 due to certified and 26.3 attributable to ordinary raw milk.

This book is of value to the person interested in the general application of control measures for the various food products. The subjects covered are so broad that the author has been forced to restrict a great deal of his information to general principles and their applications.

M.E.Hull

- 142. Standard Methods of the Division of Laboratories and Research of the New York State Dept. of Health, Second Edition.** AUGUSTUS B. WADSWORTH. The Williams & Wilkins Company, Baltimore. 1939. 681 pages, illustrated, price \$7.50.

This useful handbook of methods describes in detail the general and special technical procedures used in the various branches of the Division of Laboratories and Research of the New York State Department of Health. The assembly, under one cover, of the techniques and procedures employed in the various branches of the service provides a unique and servicable guide for the workers in similar fields.

This work is carefully prepared and well illustrated. The techniques and procedures are clearly described and easily followed.

Of special interest to the readers of the *JOURNAL OF DAIRY SCIENCE* are the sections dealing with General Laboratory Procedures; Methods Used in the Department for the Preparation of Media, Glassware, and Diagnostic

Outfits; and Methods Used in the Laboratories for Sanitary and Analytical Chemistry.

T.S.S.

## BACTERIOLOGY

143. Concerning Intestinal Bacteria. SIGURD FUNDER. *Naturen*, Nos. 7 and 8, 1939.

Mr. Funder reviews the work done in the past on intestinal microflora from the earliest days of bacteriology up to the time he conducted experiments with students at the Wisconsin University. His studies were concerned with the effect on intestinal flora in feces of adults when an exclusive diet of whole milk was used.

The experiment was carried out with three students in good health who had no previous intestinal disorders or digestive difficulties.

Contrary to previous expectations the exclusive milk diet did not change the composition of the intestinal flora. *Bacterium Coli* assumed the dominating position.

A complete milk diet did not create conditions that increased the lactobacilli in the intestinal canal. When large quantities of milk were taken with an ordinary mixed meal a decided increase in the lactobacilli was noted.

Added lactobacilli (*L. acidophilus*, *L. bulgaricus*, *L. helveticus*) increased the number in feces only during the period they were added to the diet. During this period lactobacilli were not isolated from feces. This confirms the contention that an exclusive milk diet cannot give conditions for an increase of lactobacilli in the intestinal canal. Increase in lactobacilli is obtained by drinking 2 liters milk per day. An exclusive milk diet increases the pH in feces—a reduction in acidity.

Joel G. Winkler

## BUTTER

144. Refrigeration in Butter and Cheese Making. L. C. THOMSEN, Univ. of Wisconsin. *Refrigerating Engineering*, 39: 2, 1940.

Details of manufacture of creamery butter are outlined together with necessary refrigeration requirements in connection with pasteurization cooling loads of holding and flash pasteurization systems. Short time and long time butter storage temperatures are stated. Brief descriptions are given of the three systems of cooling applied to cream; brine, "sweet" water, and direct expansion, accompanied by tables of temperature differences to be maintained. The temperatures for storage and ripening of a number of varieties of cheese together with relative humidities are listed in tabular form. A brief discussion is devoted to freezing cheese (Cheddar) and its defrosting. The cheese should be quick frozen, maintained 10° below its freezing point temperature, and defrosted in a room maintained at a temperature of 30° F. and with low relative humidity, to prevent mold development.

L.M.D.

## DISEASE

- 145. Results from Vaccination of Heifers and Calves Against Bang's Disease.** C. M. HARING, Univ. of Calif. Cert. Milk, 14: 164, 9, 1939.

The author's experiments show that the rate of recession of agglutinins induced by vaccination is fairly rapid in calves and slower in older animals, with some animals not becoming negative again for four years or more. It is also reported that there has been no evidence of the transfer of strain 19 from vaccinated to non-vaccinated cattle which have been kept together for several years.

W.S.M.

## FEEDS AND FEEDING

- 146. The Development of Dairy Heifers.** E. G. HARRISON, Cornell Univ. Cert. Milk, 14: 162, 7, 1939.

This discussion deals chiefly with two considerations. First, what is the cost of growing a heifer from birth to the freshening age? Second, is it more economical to raise or purchase the necessary replacements? The author concludes that practically every argument favors raising the necessary herd replacements. It is pointed out that the costs of growing a replacement is so great that only heifers from profitable producing dams, and sired by good bulls should be raised.

W.S.M.

- 147. Feeding of Grass Silage to Dairy Cows.** C. B. BENDER, N. J. Agr. Exp. Sta., New Brunswick, N. J. Cert. Milk, 14: 163, 5, 1939.

It is pointed out in this article that grass silage is a cheap priced high quality roughage, which has excellent production and growth values. Feeding trials showed that cows fed grass silage over the level of 45 pounds per day increased the color of milk because of the added carotene content. This milk was also found to be of higher quality as far as flavor is concerned.

W.S.M.

## FOOD VALUE OF DAIRY PRODUCTS

- 148. The Importance of a High Vitamin D Content in Milk.** H. A. RUEHE, Univ. of Ill. Cert. Milk, 14: 162, 5, 1939.

A review of the newer findings which have been made regarding vitamin D and their application to milk.

W.S.M.

- 149. Measurement of the Digestibility of Milk.** L. A. CHAMBERS, Univ. of Pa. Cert. Milk, 14: 163, 2, 1939.

The various methods which have been suggested for measuring the digestibility of milk and their significance are discussed. The author briefly summarizes the present status of this subject as follows:

"The rapid development of interest in easily digested milks has created the necessity for rapid laboratory measurement of their suitability; we have certain more or less standardized tests which measure definite properties of milk products under the arbitrary test condition; but, we do not know what properties of cow's milk or its curds should be altered to produce the desired substitute for human milk, nor the conditions under which these properties should be measured."

W.S.M.

### ICE CREAM

- 150. Gone Are the Days.** W. H. List, Jr. New York, Pennsylvania and New Jersey Ice Cream Assocs. New York, N. Y. Proc. 39th Ann. Conv. Int Assoc. of Ice Cream Mfrs., 1: 70, Oct., 1939.

This is an excellent historical presentation of the development of the ice cream industry. The author states that sanitation and pasteurization have played an important part in the development of the industry. The passage of state laws defining and regulating the product have been very helpful. The development of better distribution methods because of better highways and improved motor trucks has proved to be an impetus in the growth of the business. The product has been maintained in better condition by the use of dry ice and the development of dependable mechanical cabinets. Packaged ice creams, specialties and novelties have given the consumer a greater variety of good products.

M.J.M.

- 151. The Year's Work.** ROBERT C. HIBBEN, Sec., I. A. I. C. M., Washington, D. C. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 1: 43, Oct., 1939.

The activities of the International Association of Ice Cream Manufacturers for 1939 were largely directed towards three types of considerations.

1. Federal and state regulations.
2. Inter-industry relations.
3. Association activities.

Much effort was expended in cooperation with the Federal government in an attempt to work out satisfactory standards for ice cream. Wage and hour amendments affecting the industry were made in the Fair Labor Standards Act of 1938.

Another important function of the association work is the dissemination of goodwill and correct industry information to other industries and associations in related fields of endeavor. Much of this work has been done with the cooperation of the National Dairy Council, the Dairy Industries Supply Association and the Ice Cream Merchandising Institute.

M.J.M.

- 152. The Dairy Situation as it Affects the Ice Cream Industry.** O. E. REED, Bureau of Dairy Industry, Washington, D. C. Proc. 39th Ann. Conv. Int Assoc. of Ice Cream Mfrs., 1: 25, Oct., 1939.

The number of dairy cows in the United States increased from 1920 to 1934, then decreased until 1938. Since that time more than the average number of calves have been kept for replacement and because of this, the number of milch cows may increase during 1940 and 1941. Due to abundant feed supplies, milk production in 1938 reached an all-time high, with 845 pounds of milk produced for each person in the country. The total consumption of milk and milk products per person also reached a new high of 808 pounds of milk, or 37 pounds less than the per capita production.

Consumption of all dairy products is expected to increase. Ice cream consumption decreased 43 per cent from 1929 to 1933, but by 1937 had reached a level of 281 million gallons, which is considerably higher than that of 260 million gallons in 1929.

Factors which are retarding the development of the Dairy Industry are inefficient feeding and breeding methods, too low a price for milk and uneconomical disposal of by-products. At the present time much more attention is being given to the expansion of dairy markets and the utilization of skimmilk and whey by-products. These developments should affect the dairy industry favorably.

M.J.M.

- 153. The Ice Cream Industry in 1939.** W. J. BARRITT, Poinsettia Dairy Products, Tampa, Fla. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 1: 21, Oct., 1939.

During the past year the industry has united in working together on many pressing problems. Chief of these were in connection with the revision of the Food and Drug Act, The Motor Carrier Act and the Fair Labor Standards Act. Two tasks still ahead of the industry, according to President Barritt, are better merchandising methods and consumer education. M.J.M.

- 154. Analysis of Truck Refrigerating Systems.** O'NEAL M. JOHNSON. Int. Ass'n of Ice Cream Mfrs., Washington, D. C., Bull. 439, 1940.

In this survey, 376 ice cream plants and 48 sales branches, with a 1938 gallonage of 48,305,228 cooperated in an analysis of truck refrigeration systems. The average number of trucks per plant was  $6\frac{1}{2}$ ; however the range in number of trucks was large because small as well as large companies were included in the survey. The results of the survey are summarized in the following table:

*Ice Cream Truck Refrigerating Systems, 1939*

Type of Refrigeration	Districts					
	United States	North Atlantic	Southern	Central Eastern	Mid-west	Western
<i>Mechanical Refrigeration:</i>	%	%	%	%	%	%
Continuous: Finned coil, bare tube coil, or refrigerated wall: Condensing Unit on truck driven only during period of delivery by:						
Mechanical power take off .....	0.7	0.2	0.9		4.1	0.4
Condensing Unit on truck plugged into power line at night and driven during period of delivery by:						
Generator-electric motor system	0.9	0.3	2.8		2.4	
Mechanical power take off .....	1.9	2.1	8.3	0.8	0.7	1.0
<i>Holdover:</i> Eutectic plates pulled down or frozen by:						
Central plant ammonia system .....	11.3	7.1	33.4	7.1	21.8	7.7
Condensing unit in garage or charging dock .....	13.1	17.7	4.1	15.0	9.2	8.1
Condensing unit on truck operated only at night by hook up to power line .....	16.2	18.4	23.4	18.3	20.8	4.2
<i>Partial Holdover:</i> Combining eutectic plates and expansion coils:						
Condensing Unit on truck plugged into power line at night and driven during period of delivery by:						
Separate gasoline engine .....	0.8	1.5	0.5		0.7	
Generator-electric motor system	1.1	0.1	0.5	0.5	7.9	
Mechanical power taken off .....	1.8	0.5	4.6	2.4	6.2	0.2
<i>Non-Mechanically Refrigerated Bodies:</i>						
Dry Ice .....	35.3	36.6	5.0	37.9	1.0	64.1
Cartridge, Slug, or Bullet .....	2.8	2.1	4.1	4.1	2.7	2.6
Cartridge combined with Dry Ice .....	0.2					1.0
Ice and Salt (brine) .....	4.4	3.6	5.5	5.7	7.2	2.8
<i>Open or Panel Bodies:</i>						
Used without insulation or refrigeration .....	5.4	1.9	6.9	7.9	14.3	5.3
With "packers" .....	0.5	0.3		0.3	1.0	1.0
With "packers" using frozen brine caps .....	0.3					1.6
<i>Miscellaneous, Unclassified</i> .....	3.3	7.6				
All trucks .....	100.0	100.0	100.0	100.0	100.0	100.0

**155. A Manufacturer Speaks of Chocolate.** E. W. MEEKER, Walter Baker & Co., Inc. *Ice Cream Field*, 35: 1, 21, 1940.

The author gives the results of tests carried out under the supervision of Walter Baker & Co. comparing chocolate liquor, cocoa powder and blends of these two, all of which were made from the same blend of beans. He states that the best chocolate flavor in ice cream was derived from chocolate liquor, further, that chocolate liquor was found to give the best chocolate flavor for chocolate cake coating.

It is pointed out that these results are not wholly in agreement with previously reported findings.

It is claimed in support of these results that part of the flavor is carried in the cacao fat, hence if enough chocolate liquor is used to supply the same quantity of non-fat cacao solids that would be necessary to give a desirable flavor where cocoa powder is used, that there would be more true chocolate flavor in the first than in the second case. The author states, however, that it is possible to make satisfactory chocolate flavored ice cream with either chocolate liquor, cocoa powder or a blend of the two.

W.C.C.

**156. Sales Advance.** O'NEAL M. JOHNSON. *Ice Cream Trade J.*, 36: 1, 50, 1940.

The January-to-August Ice Cream Sales Index just issued by the Statistical and Accounting Bureau of the International Association of Ice Cream Manufacturers, shows for the first four months, a loss of 1.97 per cent had been sustained; in the second four, an increase of 5.08 per cent recovered earlier losses and recorded a gain for the entire eight-month period of 3.04 per cent. The Index is based on the reports of 779 plants, representing 1938 sales of 107,998,619 gallons.

W.H.M.

**157. Whipping Capacity of Ice Cream Mixes.** B. I. MASUROVSKY. *Ice Cream Trade J.*, 36: 1, 52, 1940.

The author reviews some of the ice cream research work of the past year giving particular attention to the article on whipping capacity of ice cream mixes by Alan Leighton and Abraham Leviton, published in *Industrial and Engineering Chemistry*, Vol. 31. Reference is made to effect of aging, homogenization pressures, butterfat content, sugar, and stabilizers on the whipping properties of the mix.

W.H.M.

**158. Successful Retail Management.** ROBERT SUTTLE. *Ice Cream Trade J.*, 36: 1, 36, 1940.

In addition to supervisory control and management, the retail store supervisor should inventory the store equipment at least every three months, investigate the background and education of prospective store em-

ployees, outline a training program for employees, hold weekly sales meetings, be responsible for the proper display of point-of-sale advertising and be alert for new merchandising plaus and ideas. W.H.M.

- 159. Quality Control of Ice Cream.** V. C. STEBNITZ. *Ice Cream Trade J.*, 36: 1, 12, 1940.

The following tests are frequently used in checking the quality of ice cream: flavor, body, texture, color, butterfat, total solids, acidity, overrun, melt-down, bacterial count, colou-aerogenes organisms, efficiency of pasteurization, and efficiency of homogenization. The author discusses each of these quality tests and how they may be used by the manufacturer in maintaining the quality of his product. The effect of ice cream ingredients on the quality of ice cream is also described. W.H.M.

- 160. Selective Distribution.** ANTHONY MENAFRA. *Ice Cream Trade J.*, 35: 12, 8, 1939.

Cardani, operating in greater New York, has found that the practice of giving selected dealers exclusive agencies in a given area has resulted in higher than average prices and has increased volume consistently. This concern has specialized on the "French" type of ice cream and a "Custom-Made" pint package which is considerably richer than their bulk ice cream. They also operate a small pastry department which makes it possible to offer dealers a special ice cream and cookie combination. Spumone is also featured by this company. W.H.M.

- 161. The Use of Dextrose in Ice Cream.** W. J. CORBETT AND P. H. TRACY. *Ice Cream Trade J.*, 35: 12, 11, 1939.

To test the adaptability of dextrose for use in commercial ice cream, all sucrose ice creams and part dextrose ice creams were compared. Results showed that the time required to secure 100 per cent overrun on direct expansion batch freezers was practically the same for both types of ice cream. The drawing temperature for the dextrose ice cream was about 1° F. lower than that of the sucrose ice cream. The replacement of 25 per cent of the sucrose with dextrose had little or no effect on the flavor and the body and texture scores. Dextrose ice cream melted more quickly in the mouth. The development of sandiness in high-serum-solid ice cream was delayed slightly by the dextrose. When dipped at the same temperature the loss was slightly greater in the dextrose ice cream; by lowering the dipping temperature of the dextrose ice cream the dipping losses were practically the same for both types of ice cream. At temperatures below 0° F. the penetration test was the same for both types, but above 0° F. the dextrose ice cream was less resistant. The time at which the dextrose was added made no difference in the whipping and freezing time. When frozen on a

continuous freezer the dextrose ice cream had a slightly sticky body; however, this defect could be corrected by reducing the gelatin content about one-fourth. Dextrose did not alter the color of the mix appreciably, lowered the viscosity of mix, generally lowered the pH slightly, and had no measurable effect on protein stability or curd tension of the mixes. The consumer preference study indicated that 1 pound of dextrose is equal to 0.83 pound of sucrose in sweetening ability. When one-fourth of the sucrose was replaced by dextrose at the rate of 1.43 pound to 1 pound omitted the consumers thought the dextrose ice cream tasted richer. It was also noted that the dextrose ice cream melted slightly faster on the tongue and gave a cooler, more refreshing effect.

W.H.M.

## MILK

### 162. The Route Profit and Loss Report and the Comparison Thereof.

H. L. GILL, Arden Farms, Inc., Los Angeles, Calif. The Assoc. Bull., Intern. Assoc. of Milk Dealers, 32nd year, 7: 182-184, January, 1940.

Particular attention is paid to the break-even point. It is stated that neither the total number of units nor dollars and cents sales are as good an indication of the merits of a route as the kinds of products sold. Individual route profit and loss accounting may cost no more than \$1.50 per month.

E.F.G.

### 163. How Odors and Flavors in Milk Can be Controlled by Feeding.

JAMES A. EMERSON, Arden Farms, Inc., Los Angeles, Calif. The Assoc. Bull. Intern. Assoc. of Milk Dealers, 32nd year, 6: 165-168, December, 1939.

Alfalfa hay should be fed immediately after milking and in such quantities that it is cleaned up within 3 hours. Green alfalfa should be fed twice daily in equal amounts immediately after milking is finished so that the longest time possible after feeding will elapse before the next milking. A full 5 hours should elapse between finishing roughage and milking. When silage is fed in outdoor racks with roughage, 10-12 lbs. daily right after milking seems to leave no harmful flavors or odors in the milk. Thirty or forty lbs. of silage at one feeding will not produce a fine flavored milk. Cows pastured on alfalfa, green barley, Sudan, sweet clover or rye must be put in dry corral 5 hours before milking to produce milk free from feed odors. Mangers not cleaned frequently are responsible for off flavors in the milk. Feed flavors in milk are controlled more by the time allowance after feeding before milking is started rather than by the kind of feed used. E.F.G.

### 164. Rancid-Flavored Milk: Its Cause and Control. N. P. TARASSUK, Univ. of Calif., Davis, Calif. The Assoc. Bull., Intern. Assoc. of Milk Dealers, 32nd year, 6: 153-160, December, 1939.

Practically all samples of raw milk contain a lipase or hydrolytic fat splitting enzyme. The lipase can commonly be activated by homogenization, violent shaking and by certain temperature changes. In the so-called "bitter milk of late lactation" rancidity develops naturally or without activation of any kind.

A Holstein cow that produced milk naturally lipase active gave milk of high activity on dry feed, but when changed to pasture failed to show any perceptible activity. Dry feed again produced lipase active milk, but this activity disappeared when the cow was placed on green feed again. Surface tension was decreased from 49-51 dynes in normal milk to 39-40 per cm. and occasionally lower for rancid milk. Rancidity can be detected by measuring the surface tension of the milk sooner than organoleptically. The addition of as little as 5 per cent of rancid milk to normal pasteurized whole milk produced an acid curd of weak clot and poor flavor. Heating the milk as soon as possible or not later than 4 or 5 hours after milking to a temperature of 130° F. for 30 minutes entirely prevented the development of rancid flavor within 7 days in the cold.

E.F.G.

- 165. Electric Dairy Utensil Sterilizers.** B. D. MOSES, Univ. of Calif., Davis, Calif. The Assoc. Bull., Intern. Assoc. of Milk Dealers, 32nd year, 5: 141-150, December, 1939.

Satisfactory heat treatment of dairy utensils according to California law requires utensil exposure to at least 170° F. water or water vapor for at least 15 minutes. Two types of equipment, one a low-pressure high-wattage, sometimes called the "instantaneous type" and the other a high-pressure low-wattage one sometimes called the "steam accumulator type" are described. These are actually accessories to steam cabinets. Two drawbacks with electrically heated boilers has been hard water scale and high electric rates. The former has been remedied through water softeners and careful operation. The latter is not easily controlled but may be comparable to boilers using oil. Advantages and disadvantages of the two types of heaters are given. Descriptions include cuts of the equipment and graphs showing relationships between characteristics of equipment and current consumption.

E.F.G.

- 166. Field Quality Control of Market Milk-Sediment Control.** WALTER F. GILPIN, Golden State Co., Ltd., San Francisco, Calif. The Assoc. Bull., Intern. Assoc. of Milk Dealers, 32nd year, 5: 135-140, December, 1939.

The author suggests doing away with a weigh can strainer—that the head on the edge of hooded pails be turned back only far enough to make a little trough which will prevent dirt from the hood entering the pail. Dust sources are drives, water, water and steam pipes and can lids (umbrella type is

recommended). The ice box is suggested as a good place to store cans. An instance is reported of an old insulated ice cream truck body being equipped with a small compressor and used as a roadside platform. In transportation a 5 ft. length of hose with a sprinkler head is suggested for washing cans. Shelter sheds are valuable for feeding hay. In one instance it was found that air currents left a dead spot at the exact location of the milk cooler allowing dust to settle. Instances of other common causes of sediment are given.

E.F.G.

- 167. Farm Control of Milk Quality.** E. H. BARGER, Borden Dairy Delivery Co., San Francisco, Calif. The Assoc. Bull., Intern. Assoc. Milk Dealers, 32nd year, 5: 129-134, December, 1939.

A careful summary is given of the factors involved in the production of high quality milk. The producer, disease in the herd, milkers, the type and care of equipment, including milking machines and the cooling of milk are treated. The important items of laboratory reports are discussed, the author concluding with the factor of producer-distributor relations.

E.F.G.

- 168. The American Dairy Science Association Quality Program for Dairy Products.** W. H. E. REID, Univ. of Mo., Columbia, Mo. The Ass'n Bull., Intern. Assoc. of Milk Dealers, 32nd year, 5: 121-128, December, 1939.

The author is chairman of the committee on quality program of the manufacturing section of the American Dairy Science Association. The organization of the committee and its functions during the past three years are outlined. The subcommittee on market milk has directed its attention to improvement of quality in the smaller towns and cities. Lack of uniformity of inspection and various interpretations of what constitute quality are found. A subcommittee on cream has made a study of the so-called French weed flavor. The subcommittee on butter has emphasized butter audits including analyses and keeping quality studies. The subcommittee on cheese has sponsored a program which includes control of many quality factors from the producer through to the final product. The subcommittee on ice cream has included educational ice cream scoring contests in its program. The subcommittee on condensed milk and milk powder has made a study of the quality control measures in 10 units in the industry. Many objectives and accomplishments of the quality program are pointed out and their significance suggested.

E.F.G.

- 169. The Relation of Metals and Their Alloys to the Flavor of Milk.** C. L. ROADHOUSE, Univ. of Calif., Davis, Calif. The Ass'n Bull., Intern. Assoc. of Milk Dealers, 32nd year, 6: 161-164, December, 1939.

Attention is drawn to the fact that exposed copper and rusty equipment

are the principal causes of the objectionable flavor of pasteurized milk. The small amounts of copper sometimes required to develop oxidized flavor is shown by the fact that at the California Experiment Station when 5 gallons of hot milk was passed through a bronze sanitary pump, the tinned surface of which had been largely removed, sufficient copper was dissolved in the milk to cause a reduction in the score of two points after 4 days storage at 40° F. Rather small quantities of copper will cause oxidized flavor when the milk is especially susceptible. Tests on a number of copper-nickel alloys revealed that those containing tin and zinc lost less copper and affected the flavor of milk less than alloys not containing these metals. Some of these alloys are good for small parts of equipment which cannot be satisfactorily fabricated from non-corrodible materials. Analyses of seven alloys are given.

E.F.G.

170. **The Production and Control of Good Flavor in Milk.** O. F. GARRATT AND C. B. BENDER, New Jersey Agr. Exp. Sta. Milk Plant Monthly, 29: 1, 23-25, 1940.

The importance of good flavor to the consumption of milk is emphasized. Placing in the hands of the consumer a fine-flavored milk is a two-fold responsibility—that of the producer and of the processor-distributor. The association between the presence of carotene and the susceptibility of the milk toward the development of the oxidized flavor suggests the necessity of feeding high carotene feeds the year round. Data show that feeds high in carotene, as grass silage, result in a milk of higher initial flavor and one more stable upon storage.

The production and feeding of grass silage appears to have several advantages: 1, the system fits into the soil erosion program through the growing of soil covering crops as legumes and grasses; 2, making of grass silage is good crop insurance as the grasses and legumes may be ensiled in wet weather; 3, ensiling grasses and legumes fit in well with the problems of producing hay; 4, the making of grass silage affords a means of making use of surplus pasture grasses; 5, ensiling grasses and legumes is a good preservation of nutrients, particularly carotene; 6, feeding grass silage results in superior flavored milk of good keeping quality; 7, grass silage of excellent quality is usually cheaper than hay of the same quality.

For winter feeding to produce milk of high color and good flavor the authors suggest the feeding of the usual grain ration, from 6-10 pounds of hay, and all the grass or legume silage the cows will eat, which ranges from 30 to 80 pounds depending on the size of the cow. They advise feeding all roughages immediately after milking, cleaning up that uneaten and ventilating the barn at least 30 minutes before each milking.

G.M.T.

171. **A Comparison of the Imperviousness of Commonly Used Paper Milk Containers.** R. B. STOLTZ AND T. V. ARMSTRONG, Dept. of

Dairy Techn., Ohio State University. Milk Dealer, 29: 2, 76-82, Nov., 1939.

To determine the imperviousness of paper milk containers five different makes of quart paper milk bottles were used. Three of the makes, referred to as numbers 1, 3, and 4, are made up and paraffined at the factory. Numbers 2 and 5 are paraffined at the bottling plant just prior to filling. Numbers 1 and 5 are of square construction, the other three makes were cone shaped.

The plan of study was as follows:

One group of containers of five makes was filled with a water dye solution and held for 72 hours at 40 degrees F. and at room temperature.

A second group of containers was filled with the dye solution, held for 18 hours at storage temperatures, then hauled around the city in a delivery truck for eight hours, and returned to storage for the remainder of the 72 hours.

A third group of containers was filled with homogenized milk and held in storage for 24 hours. The dye solution was then substituted for the additional 48 hours.

A fourth group was filled with skimmilk, whole milk, 20 per cent cream, and 40 per cent cream for 72 hours.

The final part of the study was devoted to experimenting with different waxes and methods of dipping, in an effort to produce a coating that would prove entirely impervious to moisture.

From this work the authors concluded that:

"The data collected show that there is not a paper bottle on the market today that is impervious.

Our tests indicate that the two cone shaped containers, which were made up and paraffined in the factory, offered the best protection against the entrance of moisture to the fiber. Bottle No. 4 showed the least sign of absorption of the dye solution, and the least increase in weight. This was the heaviest bottle of the lot; the increased weight apparently being due to a heavier coating of paraffin. When bottle No. 3 was given an additional dipping, increasing its weight comparable with bottle No. 4, it was then as impervious to the dye as was No. 4.

While all the bottles did not show greater increases in weight when held at the higher temperatures, there is indication that increased temperature does have a tendency to cause greater absorption.

The use of 40 per cent cream seemed to render the containers less impervious than when milk or skimmilk was used.

The average weight increases of all the containers in these comparisons were, container No. 4—0.11 grams, No. 3—0.41 grams, No. 2—0.55 grams, No. 1—1.48 grams, and No. 5—1.55 grams."

C.J.B.

172. Two-Quart Paper Containers on Retail Routes in New York City.  
ANONYMOUS. *Milk Dealer*, 29: 2, 40-44, Nov., 1939.

The Sheffield Farms Company, Inc. and the Borden Farm Products Division of the Borden Company are introducing two-quart paper milk containers on retail routes in New York City at a savings to customers of three cents per bottle. The major items of saving in the new delivery system are:

Elimination of loss from lost and broken bottles—40,000 bottles per day in our operations alone (Borden).

Elimination of time and effort required to collect and handle empty bottles—approximately 25 per cent of drivers' time—and devotion of drivers' full time to selling and delivery, with less physical effort.

Elimination of expensive bottle washing machinery and its operation and maintenance.

Elimination of ice waste in refrigeration of produce on delivery vehicles. The new container can be insulated and refrigerated far more efficiently and more economically than glass bottles.

Reduction in operating cost, maintenance and depreciation of vehicles due to lighter loads. Milk in fiber containers weighs only 40 per cent as much as milk in glass. Saving in space displacement alone is more than 50 per cent.

Finally, lower prices almost automatically bring greater consumption making it possible to effect fractional savings due to increased volume.

C.J.B.

173. Observations on Cooked Flavor in Milk—Its Source and Significance. D. V. JOSEPHSON AND F. J. DOAN, Pennsylvania State College, State College, Pa. *Milk Dealer*, 29: 2, 35-36, 54-62, Nov., 1939.

A detailed report of a study of the cooked flavor in milk. The authors report the following conclusions:

When milk, cream, skimmilk and some other dairy products are heated to a sufficiently high temperature or held for a sufficient period of time at lower temperatures, sulfhydryl compounds are formed from one or more of the proteins present.

These sulfhydryl compounds seem to be wholly responsible for the cooked flavor of heated milk and milk products. They are also responsible for the decrease in oxidation-reduction potential noted in heated dairy products, since they are active reducing substances.

The sulfhydryl compounds are active antioxidants and appear to be responsible for the inhibition of the development of tallowy or oxidized flavor in milk heated to temperatures over 170 degrees F.

In becoming oxidized (spontaneously or due to copper contamination) the sulfhydryls apparently lose their flavor characteristic and the milk or

milk product which previously exhibited a cooked flavor becomes indistinguishable from similar unheated milk.

Most heated milk products do not become tallowy or oxidized until the sulphydryls are first oxidized and the cooked flavor has disappeared. Washed cream buttermilk in some cases appears to be an exception to this general rule.

The sulphydryl substances in heated milk not only protect the milk against the development of tallowy flavor but actually act as antioxidants towards ascorbic acid (itself an antioxidant toward tallowy flavor).

Milk, cream and skimmilk heated to temperatures in excess of 170 degrees F. do not exhibit such rapid losses of ascorbic acid on storage as does raw milk or milk pasteurized in the conventional manner.

While the sources of sulphydryl compounds have not been definitely ascertained, the lactalbumin of milk seems to be the most likely ingredient responsible, with the protein of the fat globule adsorption membrane as a possible additional source. Some constituents of the milk serum, however, seem to exercise a modifying role in the reaction brought about by the heat.

C.J.B.

**174. Objectives and Accomplishments of the A.D.S.A. Quality Committees of the Milk and Milk Products Industries.** W. H. E. REID, Univ. of Mo., Columbia, Mo. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 1: 61, Oct., 1939.

The primary object of the Quality Committees of the American Dairy Science Association is to help the dairy industry to merchandise a larger volume of very high quality milk and milk products. The committee on quality are members from the faculties of the dairy departments in thirty-five states. These committees have been functioning for three years and have completed a survey of existing sanitary programs in connection with each dairy product.

The program of the sub-committee on ice cream includes the following suggestions:—formulation of standards for the ingredients used in ice cream; recommendations for sanitary control in manufacturing plants and at the point of sale; collection of statistics relative to bacteriological standards, manufacturing methods, composition, and state and municipal regulations; recommendations regarding overrun control and the preparation of a manual covering plant sanitation. Similar programs have been formulated for market milk and the principal dairy products.

The information acquired by the respective quality committees of the A.D.S.A. will be carefully studied and organized in order that it may be effectively disseminated to the industry.

M.J.M.

**175. The Antioxidative Action of Finely Milled Oat Flour on Milk.** O. F. GARRETT, New Jersey Agr. Exp. Sta. Milk Plant Monthly 29: 2, 40, 42 and 80, 1940.

A number of experiments were run to determine the feasibility of using oat flour, Avenex, to protect market milk against oxidative deterioration. Both glass and paper bottles were used in the study. Oat flour sprayed on the inner wall of the paper bottles as well as sprayed in the form of an oat oil, Avenol, onto the inner wall, while the paraffin was molten had a slight effect on retardation of copper induced oxidation. Likewise, when the paper was sized with oat flour before fabricating or paraffining the development of the oxidized flavor was retarded. By this method no oat flour flavor appeared in the milk and no weakening of the walls of the bottle was noted. Milk in similarly treated bottles exposed to the sun for 30 or 60 minutes did not develop the sunshine flavor despite the fact that only a small amount of oat flour was on the wall of the milk container and this under a paraffin film.

G.M.T.

### MISCELLANEOUS

176. **Further Development in the Modification of Cost Accounting Procedure.** E. B. McCORMICK, Assoc. Accountant, Chicago, Ill. The Assoc. Bull., Intern. Assoc. of Milk Dealers, 32nd year, 6: 175-181. 1939.

New developments in the simplified cost accounting system particularly with reference to route operating statements and break-even points are illustrated.

E.F.G.

177. **The Economic and Moral Values of Democracy.** PAUL F. CADMAN, The American Research Foundation, San Francisco, Calif. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 1: 94, Oct., 1939.

This is an able presentation of the principles and values of Democracy as it exists in America.

M.J.M.

178. **Employer-Employee Relations.** J. W. BROSTOW, Golden State Co., Ltd., San Francisco, Calif. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 1: 84, Oct., 1939.

There are four basic factors—two or more of which are always present in any employer—employee controversy. The factors are:—

Management, which wants profit.

Labor, which wants security, high hourly wages, and recognition.

Labor unions, which want to perpetuate themselves.

Government, which would eliminate oppression.

During the past few years we have seen the results of each element struggling independently towards its objective. This situation must be replaced with techniques designed to promote cooperation between the four interested groups. Rightly or wrongly, the responsibility for engineering this cooperative approach will fall upon management. If management is

tempted to decline that responsibility, before it does so, let it pause and consider what the alternatives are apt to be.

M.J.M.

179. **The Bewildered American.** W. C. MULLENDORE, Southern Calif. Edison Co., Los Angeles, Calif. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 1: 33, Oct., 1939.

This is a discussion of present economic and political questions. Suggestions are made for the solution of certain of these problems.

M.J.M.

## PHYSIOLOGY

180. **The Endocrinology of Milk Secretion.** C. W. TURNER, Univ. of Mo. Cert. Milk, 14: 160, 5, 1939.

Differences in productive ability of dairy cattle were found to be due to the difference in the rate of secretion of certain hormones of the pituitary and other glands of internal secretion. Production was increased by administration of dried thyroid tissue or injection of thyroxine into dairy cattle especially during the declining phase of lactation.

W.S.M.

181. **The Mammogenic Hormones of the Anterior Pituitary. I. The Duct Growth Factor.** A. A. LEWIS AND C. W. TURNER, Mo. Agr. Exp. Sta. Res. Bull. 310, 1939.

It had been thought for a number of years that the ovarian hormones, estrogen and progestin, directly stimulated the growth of the duct and lobule-alveolar system of the mammary gland. More recently it has been shown that the action of the ovarian hormones is indirect and that the anterior pituitary secretes a mammogenic hormone which directly activates the growth of the mammary gland. There appear to be two mammogenic factors, one which stimulates the growth of the duct system and the other the lobule-alveolar system.

This paper presents the results of studies on the duct growth factor of mammogen. An assay method was developed using the male mouse. Pituitaries were collected and assayed from 545 cattle. A low level of mammogen was found in the anterior pituitaries of fetuses, growing males and steers. The mammogen content of beef and dairy anterior pituitaries was found to be low in early and late pregnancy rising to a peak at about the 150th day. Dairy cow pituitaries contained considerably more mammogen than did those of beef cows. An even higher content of hormone was found in the pituitaries of growing heifers than in pregnant females while lactating cows had more than dry cows. During the estrous cycle the mammogen content appeared to be highest in the luteal stage.

Male rabbits given estrone responded with an increase in mammogen to double the content found in pregnant rabbits. When the estrone dosage was excessive, however, no mammogen response was secured.

Mammogen (duct growth factor) was found to be readily separated from other known anterior pituitary hormones. Any estrogen present in the anterior pituitaries was found to be far too little to have caused the mouse mammary proliferation obtained. Concentration of mammogen to 1/400 of the fresh condition was obtained. It is probable that separation of a duct-growth and a lobule-alveolar factor was secured.

Extracts of anterior pituitary were shown to develop complete mammary duct systems in male and spayed female mice, spayed female rabbits and hypophysectomized ground squirrels. Author's Abstract.

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## ABSTRACTS OF LITERATURE

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National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

### BUTTER

- 182. Fat Losses in Buttermaking.** DAVID HENRY AND WALTER SLATTER, Ohio State University, Columbus, Ohio. Nat. Butter and Cheese J., 31: 3, 12. 1940.

Studies determined that fat losses in a roll-less and in a single roll churn of the same make were identical; that pumping cream at 150° F. as compared to 90° F. increased loss of fat; and that percentage of total fat lost decreased with increasing fat content, but increased with increasing acidities of cream measured before neutralization. Losses in typical commercial plants range from 1.21 to 1.48 per cent of the total fat. W.V.P.

- 183. Buttermilk Testing.** E. W. BIRD, Iowa State College, Ames, Iowa. Nat. Butter and Cheese J., 31: 2, 58. 1940.

About 75 per cent of the fatty materials in buttermilk are true fats. Sterols and phospholipins constitute the remainder. Results are presented of twelve fat tests on a single buttermilk sample by the American Association, Babcock, Minnesota and Mojonnier tests. The first and third are recommended because they show greater spread of values as variations in fat occur. Proportion of butterfat losses are approximately calculated by:

$$\frac{(100 - 1.2 \times \text{cream test}) \times \text{buttermilk test}}{\text{Cream test}}$$

A graph is presented which shows the relation between results obtained with the different fat tests; and the relation between the per cent of total fat lost, fat in the buttermilk and fat in the cream. The graph shows that proper methods of churning recover about 99 per cent of the milk fat as butter. W.V.P.

- 184. Improving the Body of Summer Butter.** S. T. COULTER. Am. Prod. Rev., 88: 16, 462-463. Aug. 9, 1939.

The outstanding defect of summer made butter is "standing up" properties. Since this is due to a relatively high proportion of soft fats, it may be controlled by regulation of crystallization of butterfat during the processing of the cream. Control consists in cooling and holding cream at the churning temperature, this to be such a temperature that the butterfat will churn in 40 to 60 minutes; and, avoiding overloading of the churn. By such methods firmness of butter may be varied 25 per cent. P.S.L.

185. New Test for Estimated Size of Water Droplets in Butter. S. T. COULTER. Am. Prod. Rev., 88: 7, 178. June 14, 1939.

The writer reports the test devised by Knudsen and Sorensen in "Fette and Seifen." To make the test, a piece of Schleiter and Schüll No. 702 Extrahart filter paper is impregnated in a mixture of one ml. normal HCl and 100 ml. of 95 per cent alcohol containing 0.25 grams of bromophenol blue, and then dried. The paper is next held for 30 seconds against the surface of freshly cut butter. The size of the water droplets is indicated by the size of the blue spots which will form on the paper. P.S.L.

186. Effect of Salt on the Microflora and Acidity of Cream. D. I. THOMPSON AND H. MACY, Univ. of Minnesota, St. Paul. Nat. Butter and Cheese J., 31: 2, 12. 1940.

To fresh cream was added salt (NaCl) in 0, 5, 7.5 and 10 per cent concentrations. Portions were then held for 10 days at temperatures of 45° F., 60° F., and 65 to 70° F. In general the results indicate that with increasing salt concentrations in cream, the growth of bacteria and especially yeasts was checked at reasonably low temperatures. Judged by aroma, the quality of cream with 7.5 and 10 per cent salt and stored at temperatures as high as 70° could not be criticized except for slight staleness after 10 days holding. W.V.P.

187. Variability in Physical Properties of Wisconsin Butter. K. G. WECKEL, Univ. of Wis., Madison, Wis. Nat. Butter and Cheese J., 30: 12, 63. 1939.

Magnitude of variations of butter in resistance to crushing, slicing properties, "stand up" properties and "melting point" under kitchen use conditions were determined for 10 Wisconsin factories over a period of a year. Seasonal variations were of a magnitude easily observed by the consumer. Manufacturing methods may account for some variations. Tests for slicing and resistance to softening when served may be employed in creameries. W.V.P.

188. Wisconsin's Quality Improvement Campaign. L. G. KUENNING, State Capitol, Madison, Wis. Nat. Butter and Cheese J., 30: 12, 15. 1939.

The campaign is designed to "help the farmers help themselves." Farmer leaders are trained to present the program in their communities. Proper procedures are explained by experts from the Department of Agriculture and Markets and the College of Agriculture. Plant operators use methylene blue and sediment tests and report farmers delivering milk of inferior quality. State officials enforce sanitary regulations when necessary. W.V.P.

## CHEESE

189. Blending and Processing. C. R. BARKER. Nat. Butter and Cheese J., 30: 12, 14. 1939.

A successful blend should have cheese from several factories that has been slowly tempered to 70° F. The blend should be determined according to age and characteristics of the lots of cheese available. Each individual cheese should be plugged if uniformity of lots is uncertain. A good blend consists of 15 per cent current cheese; 15 per cent acid cheese at least 2 months old; 45 to 50 per cent short-held cheese (1 to 3 months of age) on the acid side but not acid; 20 to 25 per cent short-held cheese, sweet and open. Storage cheese (6 months old) can replace short-held in whole or part.

W.V.P.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

190. The Use of Different Sugars in Sweetened Condensed Skim. GEORGE J. EDMAN, Univ. of Illinois, Urbana. Nat. Butter and Cheese J., 31: 2, 15. 1940.

Effects of several sugars in the making of sweetened condensed milk are summarized. The sugars considered are sucrose, corn sugar (dextrose), invert syrup and "sweetose," a highly refined corn sugar. The solubilities of the sugars in making and storing condensed milk are compared and combinations of some sugars are recommended to eliminate crystallization. Preservative action of the sugars seems related to but not entirely dependent on osmotic pressure. Color changes in condensed milk are marked when corn sugar and invert syrup are used. Combinations of these sugars with sucrose or use of low storage temperatures or both tend to minimize this defect. Thickening of condensed is particularly noticeable when high temperatures of preheating or high storage temperatures of both are used. Corn sugar and invert syrup especially induce this change. Recommended schedules for the use of corn sugar and "sweetose" are given. W.V.P.

## FEEDS AND FEEDING

191. Kälberaufzuchtversuch mit erhitzter Vollmilch und natürlichen Vitaminzusätzen. W. KIRSCH. Züchtungskunde, 15: 1, 18-21. 1940.

At the Albertus University in Königsberg three groups of four calves each were fed from two to seventeen weeks of age on whole milk, untreated for the first group, heated for the second group, and heated but with additions of dry yeast, carrot juice and cabbage juice for the third group. The third group made the fastest and the second group the slowest gains.

J.L.L.

- 192. Untersuchungen über die Körperentwicklung, Futteraufnahme und Futterverwertung beim roten Höhenvieh.** H. VOGEL UND E. NIX. Züchtungskunde, 15: 1, 1-17. 1940.

At the University of Giessen seven heifer calves of the red highland breed were weighed each week for their first 26 weeks. Eight different measurements were taken every four weeks. Feed consumption per day and per unit of gain in live weight is given and discussed along with the growth curves. J.L.L.

### FOOD VALUE OF DAIRY PRODUCTS

- 193. Milk as a Food throughout Life.** MARGARET HOUSE IRWIN, University of Wisconsin, Madison. Wis. Agr. Exp. Sta. Bull. 447. Nov. 1939.

Science has found that milk more than any other single food meets the nutritional needs of the body. The following table shows nutritive elements in milk and human requirements:

Nutritional factor	Average daily requirement for a 154 lb. adult	Amount in 1 quart of milk	Approximate portion of the daily requirement supplied by 1 quart of whole milk
Protein	70 gm.	31 gm.	½
Calories	3000 cal.	665 cal.	½
Calcium	0.68 gm.	1.15 gm.	2
Phosphorus	1.32 gm.	0.9 gm.	½
Iron	15 mg.	2.0-5.0 mg.	½
Vitamin A	3000 to 6000 I.U.	900 to 1800 I.U.	½
Thiamin	250 to 300 I.U.	60 I.U. (raw milk)	½
Ascorbic acid	500 I.U.	520 I.U. (raw milk)	1
Vitamin D	400 I.U. (for children)	40 I.U. (Summer milk)	½
Nicotinic acid	25 mg.	Roughly 4 to 12 mg.	½
Riboflavin	1 to 2 mg.	2 to 2.5 mg.	1

Milk also contains an exceptional carbohydrate in lactose which has nutritive properties not possessed by other sugars. Lactose digests slowly, favors growth of *L. acidophilus* with consequent acid production in the intestines and favors assimilation of calcium. Milk fat is digested easily and rapidly, is needed for proper utilization of lactose and is of superior nutritive value in addition to its content of vitamins A, D and E. Effects of various processes such as pasteurization on nutritive value are mentioned briefly. The bulletin is written in a popular style and contains 40 pages, 12 illustrations, 1 table and 65 references. W.V.P.

### ICE CREAM

- 194. Controlling Distribution Costs.** O'NEAL M. JOHNSON, Int. Assoc. of Ice Cream Mfrs., Washington, D. C. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 3: 43. Oct. 1939.

In 1938 distribution costs made up 32.8 per cent of the total costs of

ice cream, according to the expense comparisons tabulated by the Statistical and Accounting Bureau of the Association. This segment of cost is almost one-third of the total and herein lies the greatest opportunity for making savings. Products costs are not susceptible to much change and neither are manufacturing costs, which are 21.7 per cent of the total costs. Distribution and sales should be watched closely and kept at a minimum, for unless sales can be made at a profit the plant cannot operate indefinitely.

M.J.M.

**195. The Status of Ice Cream Stabilizers.** W. C. COLE, University of California, Davis, Calif. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 2: 35. Oct. 1939.

This is an excellent review of the subject of ice cream stabilizers, their nature and function when used in frozen dairy products. The author summarizes the paper as follows:

"It is known that by properly proportioning the components of ice cream mixes and increasing their total solids one can produce ice cream that is palatable and smooth textured, provided the processing, freezing, and storing are properly controlled. Some manufacturers rely on this method, whereas the majority resort to stabilizers as an aid in controlling quality and as a means of facilitating the production of smooth-textured ice cream. Products such as gelatin and sodium alginate have characteristics that make them desirable for this purpose. Other stabilizers such as agar, pectin, and certain gums seem better suited for ices and sherbets than for ice cream.

"Since the properties of these stabilizers vary, it is generally possible, by selecting and using one or more of them in the correct proportion, to give the finished product certain desirable characteristics that might otherwise be lacking. This fact seems sufficient justification for their use." M.J.M.

**196. Factors Causing Shrinkage in Package Ice Cream.** ROLAND KOHLER, Arden Farms, Inc., Los Angeles, Calif. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 2: 28. Oct. 1939.

There are two types of shrinkage of ice cream in packages. One is severe and is due to a maladjusted mineral salt balance in connection with the peculiar behavior of proteins. A predominance of calcium, or a lack of proper balance between calcium and sodium in relation to phosphates and citrates, will affect the proteins, rendering them unstable. A predominance of calcium may be incorporated from low Bloom gelatin or from some other product. Subsequent high temperature pasteurization and homogenization and freezing will cause curdling of the proteins and shrinkage of the ice cream. The remedy is to adjust the mineral salt balance by adding a small amount of sodium bicarbonate to the cold, fat-free ice cream.

mix in the pasteurizing vat. Give the added product a chance to react, then subsequently add an equal or slightly smaller amount of disodium phosphate at a temperature range of 100° F. to 120° F.

The second or less serious type of shrinkage is caused primarily through neglect and inefficiency in operation of machines and too severe application of refrigeration. Excessive homogenization pressures and poor freezing are the usual causes. The result is a gradual sinking or lowering of the ice cream level from the sides and top towards the center of the can. Faulty transportation methods often are to blame for shrinkage of the ice cream. Paper packages and cans, however, seem to be as satisfactory as metal in this respect. High yield is not one of the causes of shrinkage of ice cream. If the product is made from stable ingredients, properly processed and frozen, shrinkage in the package is prevented. M.J.M.

**197. The Preparation of Frozen Fruit Pulps and Their Use in Ice Cream and Related Products.** D. G. SORBER, Assoc. Chemist. U. S. Dept. of Agriculture, Los Angeles, Calif. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 2: 7. Oct. 1939.

Since few ice cream manufacturers are in a position to obtain many varieties of fully ripened highly flavored soft fruit, it is essential that the fruit be properly preserved and stored for later use in ice cream. Such a process described in this article briefly consists of: 1. Selecting full-flavored fruit of predetermined suitable varieties; 2. Precooling as an aid to controlling oxidation; 3. Washing; 4. Coarsely crushing or pureeing (depending upon the purpose intended) in such a way as to avoid beating air into the product; 5. Adding a predetermined amount of sugar or syrup and thoroughly mixing to further aid in preventing enzymatic alteration of flavor and color; 6. Packing in tightly sealed enamel-lined tin cans, preferably closed under vacuum; 7. Rapid freezing at sub-zero temperatures; 8. Storing at a temperature of 0° F. or colder.

Rapid handling throughout the entire procedure is advisable to insure the preservation of the maximum quality that exists in the fresh fruit.

The more extensive use of frozen fresh fruit by the ice cream industry is urged as an aid to fruit growers, dairymen and ice cream manufacturers. The consumer, as well, will benefit from increased variety, greater appeal and higher quality. M.J.M.

**198. The Northwest Association's Cooperative Laboratory Project.** H. Macy, University of Minn., St. Paul, Minn. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 2: 20. Oct. 1939.

In 1938 the Northwest Association decided to: (1) adopt purchase standards for ice cream ingredients, (2) meet annually for a two-day conference of instruction, and (3) establish a cooperative laboratory to give an

opportunity for its members to have bacterial and chemical analyses made at a reasonable cost.

The standards set for the quality of ingredients to be used in ice cream are given in this article. The annual conference has proved successful and the cooperative laboratory has proven to be an economical venture. Among the various ingredients examined, gelatin has given counts exceeding the purchase standards in 66 per cent of the samples; chocolate products in 61.3 per cent; egg yolk in 47.5 per cent; nuts in 42 per cent; and strawberries in 36 per cent. All of these have frequently given positive tests for coliform types. Over 30 per cent of the colors and fifty per cent of the flavors were unsatisfactory and several candy products have been too high in bacterial content.

The results obtained to date indicate that the subscribers are making considerable progress in manufacturing frozen products which are satisfactory from the standpoint of chemical composition and sanitary quality. They are becoming more discriminating in their purchase of raw dairy products and miscellaneous ingredients. A much closer check is being made of processing and plant sanitation. So far the laboratory has proved to be a most useful agency for furnishing technical information to all manufacturers cooperating in the project. Plants having difficulty at the start are solving their problems very adequately. The future holds much promise of marked improvement when definite facts are available at all times for the enterprising manufacturer of ice cream.

M.J.M.

- 199. Merchandising Ice Cream.** JOHN C. MILTON, Hudson Mfg. Co., Chicago, Ill. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 4: 10. Oct. 1939.

This article is a discussion of the subject, "Merchandising at a Profit." The principal points in successful merchandising are discussed in an effective manner by the author.

M.J.M.

#### MILK

- 200. Significance of Coli in Milk.** H. J. BRUECKNER. Am. Prod. Rev., 88: 27, 838-839. Oct. 25, 1939.

The presence of *E. coli* in average raw milk is not significant, but if present in pasteurized milk indicates improper pasteurization or contamination after pasteurization. Recent studies at Cornell University show that less than one per cent of colon organisms survive pasteurization. Contamination after pasteurization may result from unsterilized apparatus, especially no-foam can fillers, rubber on bottle filler valves, and air tubes on bottle fillers.

P.S.L.

201. Increasing Viscosity of Cream. H. J. BRUECKNER. Am. Prod. Rev., 89: 9, 237. Dec. 27, 1939.

Two methods are outlined for increasing the thickness of bottle cream. By the first the milk, after pasteurization, is cooled to 70 to 75° F. before separation. With newer type separators temperatures as low as 65° F. may be used. Since temperatures affect richness of cream the cream screw must be adjusted accordingly. The second, more effective method, consists in cooling the milk, after pasteurization, to 50° F. or lower, holding at that temperature from two hours to overnight, warming to 80-84° F., and separating. Fat losses in skim milk will vary from 0.01 to 0.05 of one per cent and bacterial count may increase very slightly. P.S.L.

202. A Study of Oxidized Flavor. ALBERT S. TOMLINSON. Am. Milk Rev., 2: 2, 34-35. 1940.

This article is a condensed popular version of the author's thesis on the same subject. An unsuccessful effort was made to find milk which would develop oxidized flavor without addition of copper. Some samples were found developing the flavor with additions of 0.1 p.p.m. of copper. The author was unsuccessful in proving involvement of an enzyme in the reaction, although inhibition of the flavor development by high heat treatment would seem to support the theory of its role. He suggests, from results of his data, that failure of the defect to appear after high heat treatment may be due to chemical changes in the albumin of milk. P.S.L.

203. Effect of Heat on Milk with Especial Reference to the Cooked Flavor. I. A. GOULD, JR., AND H. H. SOMMER. Mich. Tech. Bull. 164. May 1939.

Studies were conducted to determine the influence of various factors upon the cooked flavor of milk, to ascertain the relationship of the cooked flavor to oxidation-reduction potentials and to oxidized flavor development, and to determine, if possible, the cause of the cooked flavor. Under the conditions of this experiment, the cooked flavor of milk occurred normally when the milk was heated momentarily to 76-78° C. This temperature was decreased with appreciable increases in the fat content, the pH, or the holding period, and upon addition of small quantities of sodium sulfite. The cooked flavor temperature was slightly increased by homogenization of the milk prior to heating, by lowering the pH, and by addition of ferrous iron at the rate of 1.4 to 2.8 p.p.m. The temperature at which cooked flavor appeared was markedly raised by the addition of one p.p.m. of copper to the milk before heating.

Decreases in the oxidation-reduction potentials were found to occur when milk was subjected to relatively high temperatures, this decrease being

closely correlated with the appearance of the cooked flavor. In addition, the heat retardation and prevention of copper-induced oxidized flavor was found to be related to the cooked flavor, especially when the copper was added before the milk was heated. The critical temperature range in this connection was approximately 84–86° C. However, when the copper was added following the heat treatment, the cooked flavor quickly disappeared and the milk became oxidized even though temperatures of 90° C. were used for processing the milk.

The liberation of sulphides from milk, probably as hydrogen sulphide, was found to be closely correlated with the appearance of the cooked flavor and the lowering of the oxidation-reduction potential. These sulphides also served to explain the effect of heat on oxidized flavor development.

Efforts to utilize the nitroprusside test as a means of detecting sulphydryl groups in heated milk were unsuccessful.

P.S.L.

- 204. Curd Tension of Chocolate Milk Drinks.** G. HADARY AND H. H. SOMMER, Univ. of Wis., Madison, Wis. Milk Dealer, 29: 3, 42. Dec. 1939.

It is shown that a low curd tension is characteristic of chocolate milk drinks. In part the reduction in curd tension is due to the higher pasteurizing temperatures used in making the products. However, the main effect is due to the cocoa itself. The effect of sugar and suspending agents such as starch, locust bean gum and "cocoloid" is negligible. C.J.B.

- 205. Processing and Handling of Coffee and Whipping Cream.** M. J. MACK, Mass. State College, Amherst, Mass. Milk Dealer, 29: 3, 38, 46–48. Dec. 1939.

The following defects of cream and their prevention are briefly discussed: 1. Poor flavor. 2. Poor keeping quality. 3. The formation of cream plug. 4. Poor body. 5. Serum separation. 6. Oiling off in coffee. 7. Feathering in coffee. Factors affecting the whipping ability of cream are also briefly discussed.

C.J.B.

- 206. Preventing Development of Oxidized Flavor in Milk.** E. O. ANDERSON, Dept. of Dairy Industry, Univ. of Conn., Storrs, Conn. Milk Dealer, 29: 3, 32, 82. Dec. 1939.

Some of the practices which are used to prevent or minimize the development of oxidized flavor in milk are listed. The case histories of five plants which have prevented the development of oxidized flavor by the use of pancreatic enzyme are given.

The author summarizes the use of the enzyme in commercial plant practice as follows: "It can be said that it offers a reliable relief from

trouble with oxidized flavor, whether the difficulty is due to metal contamination or to naturally high susceptibility of the milk. Pancreatic enzyme appears to be specific for the prevention of the development of oxidized flavor." C.J.B.

**207. The Place of Plate Heat Exchange Equipment in the Dairy.**

GLENN E. WEIST, Dairy Engineer, Chicago Ill. Milk Dealer, 29: 3, 30, 31, 78-80. Dec. 1939.

A brief discussion of the development of plate heat exchange equipment followed by examples showing how economies can be effected by regeneration. The economies are based on reduction in heating load, reduction in cooling load, and freedom from obsolescence.

In conclusion the author states that: "It seems reasonable to conclude that plate heat exchange equipment should receive consideration in all heating and cooling operations; that it offers a possibility of an improved product through processing in a closed system that is more accessible for cleaning; and finally, that it offers many striking economies in operation, flexibility plus freedom from obsolescence." C.J.B.

**208. Photochemical Study of the Irradiation Process of Producing Vitamin D Milk.** M. J. DORCAS. Milk Dealer, 29: 4, 64-68. Jan. 1940.

A discussion of what takes place when milk is irradiated. Different types of irradiators and their efficiency are also discussed. C.J.B.

**209. The Control of Sediment in Homogenized Milk.** A. J. HAHN AND P. H. TRACY, Dept. of Dairy Husbandry, Univ. of Ill., Urbana, Ill. Milk Dealer, 29: 4, 58-60. Jan. 1940.

A discussion of the causes and control of sediment in homogenized milk. The authors state that in order to eliminate sediment in homogenized milk, the following points should be considered:

If bottled homogenized milk is left standing at temperatures between 40° and 45° F. without excessive agitation, and if the milk is normal in every respect, then it will be possible to eliminate sedimentation when the milk contains approximately  $100,000 \pm 25,000$  cells per ml.

If the temperature of the milk has been allowed to increase at any time after it has been bottled, and if there has been agitation of these bottles of milk at these higher temperatures, then a cell count below approximately 50,000 cells per ml. is necessary to eliminate sediment.

Clarification or separation is necessary when milk of a cell content above 125,000 per ml. is being used for homogenizing purposes. Repeated clarification will not only reduce the cell content of the milk after each clarifica-

tion, but will permit the use of milk with a higher cell content than that required of single clarification. Clarification after homogenization is slightly more efficient than clarification before homogenization.

Destabilization of the milk protein will increase the amount of sediment while stabilization will decrease it.

C.J.B.

**210. Suggestions on Selling Milk through Vending Machines.** Anonymous. Milk Dealer, 29: 4, 44. Jan. 1940.

Two milk dealers, one of St. Louis, Missouri, and the other of Louisville, Kentucky, discuss the introduction of milk-vending machines. In large cities where drivers are unionized, they advise sticking to the half-pint bottle rather than trying the third-quart bottle. Suggestions for locating and operating vending machines are also given.

C.J.B.

## MISCELLANEOUS

**211. What's New in Farm Science.** Anonymous. Part One, Annual Report of the Director Agr. Exp. Sta., Univ. of Wis., Madison, Wis. Bull. 446. Nov. 1939.

This report presents in brief the results of work completed and in progress at this station. In the table of contents, page 95, are listed the following titles of interest to the readers of the JOURNAL OF DAIRY SCIENCE:

Animal Diseases and Breeding: Make new findings on use of hormones for breeding troubles; Does heredity control response to hormones; Determine best time for insemination of dairy cows; Is linebreeding practical with dairy cattle; High producing young cows increase production most.

Animal Nutrition: Whole milk is better than filled milk with added vitamins; Animals thrive when kept on mineralized milk only; How much would feeding grass silage improve market milk; Here's what's new in grass and legume silage; Learn more about the effects of spoiled sweet clover; Urea gives good results as a protein substitute in calf rations; Learn why animal proteins improve soybean oilmeal rations; Egg whites must contain plenty of riboflavin if the eggs are to hatch; New method cuts the cost of assaying feeds for riboflavin; Running fits in dogs can be prevented by proper diet; How do common foods compare in nicotinic acid content; Seek to isolate the grass juice vitamin; Cast new light on the value of copper in treating anemia.

Bacteria, Molds, and Yeasts: Develop quick method of producing lactic acid; Swiss cheese starter may be kept 24 hours.

Dairy Products: How closely can the fat content of Swiss cheese be controlled; What starter combinations are best for Brick cheese; Cream testing 29 per cent has advantages for buttermaking; Are propionates or propionic acid useful in buttermaking; Some treated wrappers improve

keeping quality of storage butter; Sodium alginate proves useful in dairy manufacturing; Improve test for pasteurization efficiency; Lecithin content of milk is lowest in strippings.

Farm Income and Welfare: Test of milk going to cheese factories varies considerably; Dairy cooperatives need efficient organization; Should milk be made a public utility?

W.V.P.

**212. What Shall I Use for Fuel in My Dairy Plant?** S. Konzo, Engineering Experiment Station, Univ. of Ill., Urbana, Ill. Milk Dealer, 29: 4, 50. Jan. 1940.

An arbitrary classification of the merits of various fuels and the calculated comparative costs of fuels are given.

The author then presents the following 14 methods by which maximum combustion efficiency may be obtained:

1. Provide air-tight flue passages. Seal leaks in boiler setting, smoke pipe, and chimney.
2. Provide sufficient, but not excessive draft to carry away flue gas products. Use automatic draft-regulating dampers to maintain minimum required draft.
3. Keep heating surfaces clean. Periodically remove soot accumulation from flue passages and smoke pipe. Clean out scale formation on water side of heating surfaces.
4. Inspect baffles in combustion chamber to see whether they have fallen or are leaky.
5. Maintain adequate and proper distribution of air for the combustion process. CO<sub>2</sub> percentages of the following amounts may be considered as acceptable; coal, 12 per cent; oil, 10 per cent; gas, 10 per cent.
6. Maintain smoke-free combustion. In the case of oil a slightly hazy atmosphere in the combustion chamber will usually accompany proper combustion. In the case of coal a slight trace of smokiness and a slightly yellow flame is a good visual index to use.
7. If possible, use a boiler specially designed to burn the fuel to be used. A coal-burning boiler when converted to oil burning should be properly baffled and should be equipped with special combustion chambers.
8. In all cases provide a rate of burning that is just sufficient to handle the maximum demands. Excessive burning rates may result in rapid pick-up, but also result in excessive flue-gas temperatures and large flue losses.
9. Provide adequate and trustworthy thermostatic and pressure controls for the safe regulation of the combustion process.
10. In the case of hand-fired, coal-burning plants, fire the coal into the combustion chamber so that the fresh charge does not completely cover the entire fuel bed. Live coals should be exposed so that volatile gases will ignite. Remove ashes from the ash-pit to prevent burning out of the grate bars. Egg-sized and nut-sized coals should be used.

11. In the case of stoker-fired plants, remove clinkers periodically from the fuel bed. Stokers will not satisfactorily handle any old coal. Best results are obtained with coals specially prepared for stoker use. This preparation includes sizing (1-inch, 1½-inch or 2-inch coals are used, depending on the size of the stoker), washing, and oil treating of the coal.

12. In the case of oil-fired plants use clean oil of the quality recommended for the specific burner. Periodic and frequent inspections of the oil strainer, burner nozzle, and ignition device should be made.

13. In the case of gas-fired plants, provide proper and constant gas pressure, reliable pilot lights, and fool-proof safety controls. Periodic inspection of the chimney should be made to determine whether any flue-gas condensation may be damaging the chimney or smoke pipe.

14. Provide boiler plants with draft gauges, flue-gas-temperature recorders, and some means for checking the CO<sub>2</sub> in the flue-gas, either periodically or intermittently.

C.J.B.

**213. Internal Audit Control.** CHARLES W. TUCKER, H. P. Hood & Sons, Boston, Mass. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 3: 54. Oct. 1939.

Mr. Tucker summarizes his discussion by stating that a satisfactory state of internal audit control may be said to have been achieved when the factors of organization, personnel, policies, and procedures, records and mechanical and other equipment aids have been so developed and combined as to produce a harmonious whole. This coordinated effort should make possible an "Earnings Account" with a credit balance commensurate with what management and stockholders might reasonably expect as a return on their investment of effort and capital.

M.J.M.

**214. Marquis of Queensbury Rules for Modern Business.** I. E. LAMBERT, Attorney at Law, Santa Fe, New Mexico. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 3: 28. Oct. 1939.

This is a discussion of trade practices, which in the opinion of the author, hinder business. In addition he discusses possible future legislation which should be followed with keen interest in order to see that it is practicable and workable.

M.J.M.

**215. Executive Use of Accounting.** PAUL H. ANDRES, The Central Dairy Products Co., Oklahoma City, Okla. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 3: 8. Oct. 1939.

The writer states that the responsibility of an accounting department is to give to management what it needs and wants. Management should accept accounting reports as accurate statements of facts, containing the

clews to bad operating and other conditions of business. If the accounting department is competent, and if management realizes the functions of accounting and is willing to use all the information it gets, then the executive and accounting departments will continue the progress already made, and better operating results will be deprived from these efforts. M.J.M.

216. Why Sales Are Lost. FRANK WARREN, Arden Farms, Inc., Los Angeles, Calif. Proc. 39th Ann. Conv. Int. Assoc. of Ice Cream Mfrs., 4: 22. Oct. 1939.

There is a reason for losing sales, and when the cause is known a remedy may be found. Sales are lost because of indifference, delay in serving the customer, mistakes, worry, ignorance, a poor quality of merchandise, lack of cleanliness, lack of personality, ungratefulness, and price. The author considers each of these causes separately and offers solutions for preventing loss of sales by any of these factors. M.J.M.

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## ABSTRACTS OF LITERATURE

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Columbus, Ohio

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New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

### BACTERIOLOGY

- 217. Chromorésistance et Enrobage Phosphocalcique des Microbes Chauffés dans le Lait (2). (Resistance of Staining and Phosphocalcic Enrobing of Organisms Heated in Milk.)** M. G. GUILTONNEAU AND M. BEJAMBER. *Le Lait*, 19: 225. March, 1939.

In a former study, it was shown that certain micro-organisms displayed a definite resistance to staining after being subjected to heat in a milk medium. This "white image phenomenon" could be produced by heating the organisms in raw skim milk for one hour at 100° C. or by a similar temperature treatment in a medium of calcium caseinate and calcium phosphate at pH 6.7.

A strain of *Str. thermophilus* was used in the present study. After being heated in milk for one hour, the bacteria displayed the stain resisting property. This could not be altered by washing the organisms in decinormal alkali but it was removed by dilute acids and CO<sub>2</sub>. Micro-analytical methods applied to a suspension of the enrobed cells indicated that the covering was probably Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> absorbed on the outer cell membrane. O.R.I.

- 218. Les Formes S et R Des Colonies Chez Les Colibacilles. (S and R Forms of *Bacillus Coli* Colonies).** IRENE LIPSKA, Municipal Institute of Hygiene, Warsaw. *Le Lait*, 19: 1016-1027. 1939.

Interest had centered for some time on the types of colonies produced by colon organisms. The causes of variations and the difference in virulence of the two forms is not yet fully understood. The author reports that most natural sources—milk, butter, feces, pathological urines—produce the S form in the greatest numbers but that the majority of colonies from cheese were of the rough colony type.

Using Endo's agar at 37° C. and carbohydrate broths, a study was made of several factors affecting colony types. Drying of the agar surface of plates caused the R forms to revert to the S form. Low incubation temperatures favor this change. The addition of gelatine and of bile to Endo's agar favored the growth of the R and the intermediate forms. The R form when so obtained in the laboratory is not stable however.

There is little or no difference in the reaction of the two forms to the action of bacteriophages. When new cultures were used for determining this activity, the phage dissolved colonies of its own form more rapidly than those of the other type of colony. O.R.I.

- 219. Recherches sur les Bactéries Propionique (Studies on the Propionic Bacteria).** W. DORNER. *Le Lait*, 19: 897-918. 1939.

This paper is an important contribution to our knowledge of Swiss cheese

cultures. Examinations were made of the milk received, and of the rennet and starters used in many of the cheese plants in Switzerland. Counts were made on samples by heating them to 58° F. for five minutes and determining the surviving organisms capable of growing on a yeast extract-peptone-sodium lactate-agar medium.

Some sources of milk were found to have comparatively high counts of propionic bacteria. In most cases rennet and ripened whey were not important sources. Examinations of aseptically drawn cow's milk show comparatively high counts in many cases.

Work is reported on the effect of propionic bacteria cultures upon eye formation and density. In most cases eye formation was increased where light inoculations were employed. Heavy inoculations increased the content of propionic acid bacteria in cheese but did not favor eye formation. The final specific gravities of inoculated and control cheeses were the same at the end of three months although the desired condition was brought about earlier in the case of the inoculated cheese.

Propionic bacteria were found to consist of two types; cocci and rods. Forty-five strains of the coccus form were isolated and their cultural characteristics determined. By holding these on agar slants for 30 to 45 days at 30° C. the cocci gave rise to the rod forms. No means was found of reverting rods to cocci however.

Most strains of cocci grew best at 30° C. and had maximum growth temperatures of 42–50° C. Both propionic and acetic acids were produced, usually in the ratio of 2:1. Milk was coagulated and the following carbohydrates hydrolysed: glycine, levulose, dextrose, mannose, galactose, sucrose, maltose and lactose. Gelatin was not liquefied. O.R.I.

## 220. Microscopic Examination of Dairy Products and Their Calculations.

HANS EDEL, Gehl's Guernsey Farms, Milwaukee, Wis. Milk Dealer, 29: 5, 34–35. Feb. 1940.

The author presents a chart which eliminates any figuring when using the Breed smear method for determining the number of bacteria in dairy products. C.J.B.

## BUTTER

### 221. Neutralizing Cream for Buttermaking. I. A. GOULD AND R. C. TOWNLEY. Can. Dairy and Ice Cream J., 19: 2, 48. 1940.

Soda neutralizers were found to be more efficient than limes in lowering the acidity of butter and butterfat. The acidity of butterfat was found to be lower than that of the butter but the extent of the difference varies somewhat with the type and amount of neutralizer used. The degree of neutralization of each batch of cream needs careful consideration.

O.F.G.

**222. The Deterioration of Butter in Storage.** W. C. CAMERON. Can. Dairy and Ice Cream J., 19: 2, 22. 1940.

Most of the flavor defects in butter are probably due to either bacterial or chemical action in the cream or butter. The extent to which these flavor defects occur in butter can be materially reduced by: (1) withholding from churnings intended as first grade all cream showing unclean or stale flavors regardless of the acid content of the cream; (2) sanitary conditions in the creamery being measured by frequent and systematic mold analyses, and necessary control measures as indicated by the results of such analyses being applied; (3) giving due consideration to the type of package used and the temperatures at which butter is held immediately after churning and until entering storage for long holding.

O.F.G.

**223. The Creamery Industry under War-Time Conditions.** C. E. LACKNER. Can. Dairy and Ice Cream J., 18: 12, 47. 1939.

Under war-time conditions the disposition of surpluses is not a problem of Canadian butter manufacturers but there is the problem of increased production to meet a greater demand. The problem is really one of increased milk production rather than a shift in amount from one type of product made from milk to another. The methods suggested for bringing about increased production are first, increasing the number of cows in the herd, and second, increasing the yield per cow.

O.F.G.

**224. Butter—A Vital Food.** W. A. SPROULE. Can. Dairy and Ice Cream J., 18: 12, 15. 1939.

This is partly a discussion of the importance of butter in a war economy. The importance of butter in the human diet especially from the standpoint of fat digestibility and vitamin A and D content is emphasized. Butter is prized above most other edible fats because of its superior flavor but it is not only flavorful in itself, but adds to that of other foods consumed with it. There is no butter substitute acceptable by people who realize the true value of butter if the finest quality is available.

O.F.G.

**225. A Discussion of Butter Working Methods.** H. McNEVIN. Can. Dairy and Ice Cream J., 18: 10, 45. 1939.

This article emphasizes the importance of treatment of a new churn barrel. The inside diameter should be checked to be sure the sides are parallel and to be sure that the churn is level. Lime, washing compounds or acids should never be used but scalding hot water should be employed in cleaning the churn.

O.F.G.

226. Cow Butter. B. F. McKIBBEN. Pacific Dairy Review 44: 3. 1940.

The southern housewife often calls home-made butter "cow butter," while creamery butter is considered second grade.

As flavor is the basic factor for determining the quality of butter, the housewife's distaste of the manufactured product may be due to the flavor of the water used in the creamery. Numerous off-flavors, such as the "cheesy" and "oily" flavors, may be caused by impure wash water.

V. L. Turgasen of Armour and Company reached the conclusions that water supplies from rather widespread areas are capable of bringing about certain objectionable changes in butter, and that some chlorinated water may not be satisfactory for butter-making purposes. The use of absolutely clean water in making butter is a fundamental of great importance and should receive greater attention.

P.A.D.

227. Sur Les Beurres Anormaux (On Abnormal Butters). P. BALAVOINE, Le Lait, 19: 1027-1030. 1939.

This paper is a review of recent reports which deal with the significance of the Reichert-Meissl number and the refractive index as measures of butter purity. Such factors as changes in feed, stage of lactation, stable and atmospheric temperature, breed, etc., are suggested as causes of variation. An early hypothesis is revived that the source of butyric and other low boiling point fatty acid radicles present in milk, is the fermentation that takes place in the rumen of cattle.

O.R.I.

## CHEESE

228. Notes on the Preparation and Action of Rennet. J. G. DAVIS. Can. Dairy and Ice Cream J.. 19: 2, 56. 1940.

The rate of rennet clotting increases rapidly with small increases in the acidity of the milk. Albumin and globulin retard coagulation which is one of the reasons why mastitis milk clots slowly with rennet. Boiling the milk previous to adding the rennet removes the inhibitory effect. A rapid decline in rennet "coagulability" is observed after milk is taken from the udder. Desirable qualities in a good rennet are (1) constant strength, (2) good keeping quality, (3) freedom from fault-producing micro-organisms, and (4) freedom from other enzymes. Most samples of commercial rennet contain other enzymes but these should be kept to a minimum.

O.F.G.

229. Studies of Starters for Cheesemaking. E. G. HOOD. Can. Dairy and Ice Cream J., 19: 1, 51. 1940.

The power to produce acid steadily throughout the cheesemaking process and at regular intervals from day to day is the most important property of

a good starter. In addition the culture should have good flavor and aroma. A small scale test for determining the vitality of cheese starters is described and the following precautions are given: (1) Use clean jars which have been sterilized, (2) Use one batch of milk for filling all the jars, (3) Stir the mother culture well before taking sample, (4) Measure 5 cc. starter accurately, (5) Measure 1 cc. of rennet accurately, (6) Mix both starter and rennet well with the milk, but do not overstir the rennet, (7) Cut the curd in all the jars to the same degree of fineness, (8) Keep the temperature of the bath constant at 100° F., (9) Drain the whey thoroughly at each stage and to an equal degree from each jar. The vitality test has the ability to differentiate between the acid-producing powers of starters when tested under similar conditions.

O.F.G.

**230. Cottage Cheese.** D. W. GLOVER. Ohio State Univ., Milk Dealer, 29: (6), 42. 1940.

A brief discussion of the influence of the following factors on the quality of cottage cheese:

1. The quality of the raw milk.
2. Pasteurizing temperatures.
3. The quality and amount of starter.
4. The amount of rennet enzyme used.
5. The acidity of the whey at the time the curd is cut.
6. The use of water to aid in cooking.
7. The rapidity of heating the curd.
8. Time and temperature used in cooking.
9. Temperature of wash water and number of washings.
10. Proper chilling of the curd before creaming.

The author also reports that homogenized milk returns may be utilized in the production of cottage cheese by the addition of calcium chloride, 1.0 cc. of a saturated calcium chloride solution per 100 pounds of milk being sufficient to restore the coagulating properties.

C.J.B.

**231. Les Formes Levures dans la Flore Superficielle des Fromage de Camembert. (Yeast Types in the Surface Flora of Camembert Cheese).** G. GUITTONNEAU, J. KEILLING AND H. DE LAVAL. Le Lait, 19: 338. 1939.

The organisms which produce the surface covering of Camembert cheese are thought to be very necessary for good flavor development. Samples of the surface slime were taken at intervals during the first 12 days of ripening from cheese manufactured in two Normandy factories. The flora were comprised of yeasts almost entirely with a few mycoderm and *Ödium* colonies. The yeasts were largely of the *Torula* type.

A cultural study of the yeasts present showed that they were capable of digesting protein, fermenting sugar, and neutralizing acidity. Most strains

grew well on lactose, saccharose, glucose and levulose, some producing large amounts of alcohol, volatile acids and esters. A great majority of these strains were resistant to high concentration of salt and lactic acid.

O.R.I.

**232. L'utilisation Économique du Froid Artificiel dans la Conservation et L'amélioration des Fromages à la Pâte Persillée. (The Economic Use of Refrigeration in the Holding and Curing of Mould Cured Cheese).** A. MOULIN. *Le Lait*, 19: 924-926. 1939.

Significant improvement can usually be affected where refrigeration is available for the curing of cheese of the Roquefort type. Two experiments are reported in which temperatures of 4-6° C. were employed. One part of a batch of cheese was tin-foiled and held at this temperature while the second portion was left unwrapped. Improved flavor, sharper color, more uniform texture and a lower loss from surface growth resulted in the case of the wrapped cheese. Earlier wrapping is possible where refrigerated storage is available.

O.R.I.

**233. Dosage de la Matière Grasse dans les Fromages. (Determination of Fat in Cheese).** HENRI COUTURIER. *Le Lait*, 19: 918-924. 1939.

Three methods of determining fat in cheese—volumetric (Gerber), direct extraction (Soxhlet), and indirect extraction (Schmidt)—are discussed from the point of view of accuracy and convenience. The author stresses the need for more fully standardized methods of sampling and also the fact that fat percentages vary with losses in moisture. The Schmidt method in which the solids-not-fat are first digested by hydrochloric acid possesses distinct advantages and can be rapidly carried out providing tared glassware is available to allow rapid weighing. No experimental values or comparisons are given however.

O.R.I.

**234. Les Fromages à la Crème. (Cream Cheese).** J. M. ROSELL. *Le Lait*, 19: 698-703, 811-814. 1939.

Manufacturing methods for several types of cream cheese common in Europe and America are presented in this review. Among the types discussed are the following: Double cream, sweet cream, English simple cream, Gervais cream, Fontainebleau, Italian or Mascarpone, Neufchâtel, Philadelphia and pasteurized cream cheese.

O.R.I.

**235. Théorie de la Maturation des Fromages Durs (Theory of the Curing of Hard Cheeses).** S. ORLA-JENSEN. *Le Lait*, 20: 2-16. 1940.

In a copyrighted feature article, the author traces the development of our knowledge of the biochemistry and bacteriology of cheese curing through the years, paying special reference to Swiss cheese.

Duclaux, the first director of the Pasteur Institute pioneered in this field and his work was followed by that of the Swiss bacteriologists Freudenberg, by Orla-Jensen and by the Austrian, Ademetz. As a result of studies made on "natural" rennet at this time, Christian Hansen began the manufacture of rennet commercially in Denmark in 1874.

Rennet was shown to be a source of proteolytic enzymes and the effect of acidity on proteolysis became the subject for study shortly after Sörensen developed his methods of determining hydrogen-ion concentration. The relation of proteolysis and pH to texture, first demonstrated by van Slyke, was an important contribution during this period. Extraction of the juice of cheese by pressure, in order to determine protein degradation was reported first in 1929.

Present-day knowledge of the volatile acids produced by lactic acid bacteria is based largely on the authors' studies. The isolation and culturing of propionic acid bacteria in calcium lactate media has opened the way for much research on Swiss cheese problems.

O.R.I.

**236. Propionibacterium Rubrum from Dairy Cheese.** LUBON A. MARGOLENA AND P. ARNE HANSEN, Royal Technical College, Copenhagen. Reprint, Zentr. Bakt. II, 99: 107-115. 1938.

*Propionibacterium rubrum* van Niel has been isolated from a dairy cheese in which distinct colonies had been formed. A description of the species and pigment production is given.

The culture can be separated by means of the centrifuge in a pigmented and an unpigmented portion. The pigment is insoluble in the ordinary fat solvents, and is not, as previously assumed, a carotenoid. It is of no importance in the oxygen uptake of the organism.

L.H.B.

## CHEMISTRY

**237. Recherches sur L'état Physicochimique des diverses Substances Lipoidiques du Lait et Particulièrement des Phosphatides et du Cholestérol (Studies on the Physicochemical State of the Different Lipoid Substances in Milk and Particularly of the Phosphatides and of Cholesterol).** F. TAYEAU. Le Lait, 20: 129-134. 1940.

Other investigators have shown that the addition of sodium or potassium soaps to blood serum results in the liberation of additional fats not recoverable by direct ether extraction. These fats are present in the blood serum as part of the lecithin protein fraction.

This method has been adopted for the study of milk. The ether extracts are washed with distilled water and the ether then evaporated. After the weight of the residue has been determined, aliquots are analysed for phos-

phorus and for cholesterol, the latter determination being made by a colorimetric method.

The addition of small amounts of 20 per cent sodium linoleate solution to the milk at the time the ether was added, increased the efficiency of the extraction in the case of all three fat fractions—total fat, phosphatide and cholesterol. As the amount of soap solution was increased above 2.5 ml. per 100 ml. of milk the efficiency of extraction fell off rapidly. However, the curves for all three fractions were very similar. The author suggests that without the use of a soap solution in the extraction, approximately one-tenth of the total fat, three-tenths of the phosphatide and three-tenths of the cholesterol, are not extracted by ether alone.

O.R.I.

- 238. Sur L'ammoniaque du Lait. (On the Ammonia of Milk).** J. HELIER AND W. SWIECHOWSKA, Univ. of Wilno, Cracow, Poland. *Le Lait*, 19: 1009-1016. 1939.

A method originally devised for the determination of ammonia in blood has been adopted by the above authors for the determination of ammonia in milk. Sodium borate is added to the sample, the ammonia is distilled off under reduced pressure, and the amount calculated from the Nessler reaction read in a photelometer. Fresh milk samples may be held for some time if the surface is covered with paraffin oil.

The source of significant increases in ammonia was found to be the result of the growth of micro-organisms, the amount present being an indication of the microbial content. The data presented indicate, however, that no great increase takes place in ammonia content until the bacteria count reaches figures in the millions.

O.R.I.

- 239. Sur L'identification de la Grasse de Coco par le Methode du Dr. L. Hoton (The Identification of Cocoa Fat by the Method of Dr. L. Hoton).** F. LIVARI, C. MANTOVANI, AND E. TURCO, Laboratory of Hygiene, Parma, Italy. *Le Lait*, 19: 785-798. 1939.

The accurate identification of cocoa fat as an adulterant in butter is very difficult. Dr. Hoton of Belgium suggested the use of the refractive index of the insoluble volatile fraction obtained in the Reichert-Polenske distillation as an aid in its detection. In the formula

$$Q = \frac{VSA \times R.VIA}{10 \times VIA}$$

where values of Q equal or exceed 20, the absence of cocoa fat is indicated. Values lower than 15 indicate its presence. VSA refers to volatile soluble acids, VIA to volatile insoluble acids and R.VIA to the Zeiss index at 25° of the volatile insoluble acids.

The authors show that the method possesses disadvantages and suggest the use of the ratio  $\frac{R.VIA}{VIA}$  in conjunction with the VSA value as a means

of analysis. Abnormal values due to feed are discussed and the failure of the method in the case of sheep's and goats' milk cheese is also shown.

O.R.I.

## DISEASE

- 240. The Importance of Vitamin A in Animal Life and the Effect of Its Deficiency upon Animals.** G. H. HART, Univ. of Calif., Davis, Calif. The Assn. Bull. Intern. Assn. of Milk Dealers. 32nd year: 13, 303-314. Feb. 1940.

Chlorophyll, carotene and xanthophyll, the several forms of carotene, and their relationship to Vitamin A are discussed. The storage of Vitamin A in fish livers from their phytoplankton diet is slow and in proportion to the age of the fish. An early experience with rancidity in milk due to Vitamin A deficiency is reported. The problem of determining the vitamin A value of a diet is complicated by the fact that a part of the vitamin A is preformed and a part consists of carotene transformed to vitamin A in the animal's body. The former is three times as effective per microgram as the latter so the proportion of each in the ration would need to be known. The unfavorable color effect of carotene upon meat fat and egg yolk is mentioned. Vitamin A deficiency results in temporary sterility and the birth of dead or weak calves. This is a very excellent discussion of the importance of vitamin A in animal life.

E.F.G.

- 241. Vitamin A Deficiency in Man—Its Consequences and Methods of Detection.** HAROLD JEGIERS, Boston Univ. School of Med., Boston, Mass. The Assn. Bull. Intern. Assn. of Milk Dealers, 32nd year. No. 13, 289-302. 1940.

The history of vitamin A is traced and its relation to night blindness, Xerophthalmia and infections shown. The better dietary sources of vitamin A are dairy products, liver, eggs and the highly pigmented vegetables and fruits. An instrument devised by Dr. Jeans measures night blindness which is caused by an impairment of the ability of the eye to replenish visual purple destroyed by bright light. Vitamin A deficiency in humans is tested by measuring this ability of the eyes. The Hecht and the Edmunds methods are also described. It is reported that students receiving 3,000 to 4,000 I. U. of vitamin A daily apparently had normal eye adaptation. Night blindness was caused experimentally by reducing the daily intake of vitamin A to 1/10 to 1/20 of the normal requirement. In eye impairment, the cone cells as well as the rod cells are affected, but not to as great an extent. A detailed case study is given of a student who must receive vitamin A from day to day as he could store only subnormal quantities in his body. This student given 100,000 units of vitamin A showed improvement

in night blindness in 40 minutes and at the end of 2 hours was normal. Three or four days served to render him subject to night blindness again. Persons deficient in vitamin A are also invariably subject to dazzling by bright lights. Adequate quantities of vitamin A for adults is given as 2,000 to 4,000 units and for children 6,000 to 8,000 units. A clinical procedure for diagnosis of vitamin A deficiency is given.

E.F.G.

**242. The Detection of Abnormal Cow's Milk by Microscopic Methods.**

S. HADWEN AND R. GWATKIN. Can. J. Res., 17: Sec. D, 225-244. 1939.

In an attempt to establish standards for normal milk and furnish a basis for the more accurate diagnosis of mastitis, Breed and sediment smears were made on a large number of cows' milks. More information was obtained from the sediment count especially when the types of cells found were taken into consideration.

In staphylococcal mastitis the leucocytes are often very numerous, appearing as large, ring-shaped, polymorphonuclear cells. To a certain extent the resistance of the cow and the stage of infection can be estimated from the proportions of leucocytes and cocci present.

In streptococcal mastitis the mononuclear leucocytes are numerous, often in loose irregular clumps. In most cases leucocytes and cocci are not as numerous as in the staphylococcal type of infection.

The diagnosis of *B. coli* and *Corynebacterium pyogenes* mastitis is discussed as well as other phases of abnormal milk secretion including the significance of red blood cells, chromatin-staining granules and calcium concretions.

O.R.I.

## ICE CREAM

**243. Reclaiming Butterfat from Homogenized Milk Returns.** R. W. GREENWOOD. Can. Dairy and Ice Cream J., 18: 12, 57. 1939.

A processing temperature of 145° F. appears to give the greatest efficiency of homogenization, pressure being constant, with the best possibilities for fat reclamation. Checks on the fat loss when homogenized milk was separated alone showed 1.1 per cent. Mixing homogenized milk with unhomogenized milk up to 20 per cent previous to separation does not merely spread the fat loss over the whole volume, but also improves the reclamation of the homogenized fat by approximately 20 per cent. The cream obtained from this procedure can be used in churning, and practically normal churning of butter will be obtained.

O.F.G.

**244. Ice Cream Sales Index.** ANONYMOUS. Special Bull. 63, Intern. Assn. of Ice Cream Mfrs., Washington, D. C. April, 1940.

This bulletin contains an analysis of ice cream sales for the year 1939, in comparison with the previous year.

The 1939 wholesale sales of ice cream increased 5.59 per cent over 1938. This means that 1939 was the biggest year in the ice cream industry, with sales exceeding 1938 by fourteen and a half million gallons, and 1937 by approximately 2,700,000 gallons.

The bulletin contains the ice cream production figures by states and sections of the country.

A supplement to the bulletin also gives monthly variations in production and employment. The decrease in production during the winter months is more severe than the decrease in employment. For example, December production is only 28 per cent of the July production while employment in December is 59.4 per cent of the July level.

M.J.M.

**245. Insulated Bags for Carry-Home Packages of Ice Cream.** ANONYMOUS. *Ice Cream Rev.*, 23: 6, 42. 1940.

The Ice Cream Review conducted a survey to determine the extent to which insulated bags are used to protect carry-home sales of packaged ice cream. It was found that approximately 31 per cent of the wholesale manufacturers supply their dealers with the bags, while 54 per cent of the retail ice cream manufacturers use such bags. Other results of the survey are cited.

J.H.E.

**246. Lignin Vanillin Comes from New Source.** ANONYMOUS. *Ice Cream Field*, 35: 3, 53. 1940.

It is pointed out that lignin, derived from spruce and other conifers is now being used commercially as a source of vanillin. The two main sources of synthetic vanillin have been (1) the so-called guaiacal vanillin synthesized from benzol derivatives and (2) the so-called eugenol vanillin obtained from clove oil or cinnamon leaf oil.

The General Drug Company reports that the new extraction plant which has been operating less than two years already supplies about 50 per cent of the vanillin now being used in the United States. According to the report lignin vanillin is a product of high purity and is used widely by flavor and extract manufacturers that supply the ice cream, candy and baking industries.

The main steps in the process of manufacture of lignin vanillin are briefly outlined.

W.C.C.

**247. Dry Ice Truck Refrigeration.** E. M. WESTBERG, Refrigeration Consultant. *Ice Cream Field*, 35: 3, 35-39. 1940.

The author states that analysis shows that dry ice as an ice cream truck refrigerant has consistently gained ground, despite the fact that various mechanical systems have made marked progress. Mechanical and dry ice

systems have gained chiefly at the expense of ice and salt and cartridge systems. W.C.C.

**248. Quality Control, Part II.** V. C. STEBNITZ, Chicago Dairy and Food Laboratories. *Ice Cream Trade J.*, 36: 2, 26. 1940.

Part II of this discussion deals with the acid content of the ice cream mix, maintaining uniform composition, and the use of various non-dairy products constituents.

If neutralization is to be done it should never be carried to the extent of reducing the acidity below the normal acidity of the mix (about .19 per cent for a 10.5 per cent serum solids mix). The acid test as a measure of quality or freshness is limited by the fact that bacterial growth must take place to an appreciable extent before enough acid will be produced to materially affect the titratable acidity test.

It is important to test the mix for fat and solids. Although it may be more convenient in some plants to measure rather than weigh the liquid ingredients, more accurate results are obtained by weighing.

Although non-dairy ingredients do not spoil readily, their proper care is important in maintaining a low bacterial count. Quality control requires constant vigilance, not only from the control laboratory but for every one connected with the operations from the procurement of the raw material to the delivery to the consumer.

W.H.M.

**249. Practical Production.** CHARLES POLICASTRO, Abbotts Dairies, Philadelphia, Pa. *Ice Cream Trade J.*, 36: 3, 26. 1940.

The following problems encountered in ice cream plant operation are discussed: (1) use of homogelized products for standardizing mixes; (2) the staggering of ice cream containers in the hardening room to provide proper air circulation; (3) selection of flavors; (4) use of dairy products of low natural acidity; (5) proper operation of the freezer; (6) importance of sanitation; (7) labor efficiency that will insure proper number of man-hours per unit of product; (8) elimination of waste and (9) proper planning of work, to make possible uniform power consumption. It is the author's belief that the ability of the plant operator to produce the largest amount of quality material per man-hour requires more than formulae and ingredients, it requires in addition to these, proper planning, cooperation of employees, alertness, and selling of ideas to the plant employees.

W.H.M.

**250. Freezing-point Data of Corn Syrup Solids.** A. P. HELLWIG AND B. F. BUCHANAN, Technical Service Laboratories of the American Maize Products Co. *Ice Cream Trade J.*, 36: 2, 49. 1940.

Data is presented to show that solutions of corn syrup solids (Fro-Dex) have a higher freezing point than sucrose or dextrose solution of similar con-

centrations. The corn syrup solids is reported to contain dextrose, maltose, and edible dextrins in the approximate ratio of 15:43:42. W.H.M.

**251. Factors in Low Temperature Refrigerator Bodies.** T. J. HACKNEY, Hackney Brothers Body Co. *Ice Cream Field*, 35: 2, 56. 1940.

The author outlines the requirements of good insulation and then discusses the more common systems of refrigeration in use on refrigerated trucks. He indicates that:

1. Where dry-ice is available economically, it provides the desired refrigeration at a minimum weight of apparatus or equipment.
2. Hold over plates are most economical when piped to a source of refrigerated gas through make and break valve connections. This system is suitable if the truck has a sufficient rest period between loads.
3. Bodies equipped with Freon or Methylchloride compressors permanently hooked-up with the hold-over plates are more satisfactory when the truck does not return to the plant each night, but will be where the desired power is available.
4. With exceedingly long runs and uncertain destinations continuous refrigeration may be taken from the truck chassis or may be obtained by a separate gasoline motor. The use of hold-over plates in a system of this kind may also be desirable.

The author claims that by the application of the above methods of refrigeration it is possible to solve the problems of low temperature truck operation.

W.C.C.

**252. Greater Plant Efficiency through Better Supervision of Delivery Equipment.** E. A. KAYSER, St. Louis Dairy Co., St. Louis, Mo. *Ice Cream Field*, 35: 2, 12, 13, 58, 59. 1940.

It is pointed out that delivery expenses of ice cream, exclusive of merchandising costs, amount to 56.8 per cent of total expense, whereas, it amounts to 34 per cent of the ice cream sales dollar.

With large scale operation he outlines the advantages in contracting for such items as gasoline, lubricating oils and greases, and tires.

He gives detailed instructions as to "the care of tires" and "garage technique," and reproduces the "Truck Preventive Maintenance Record" used by the St. Louis Dairy Company.

W.C.C.

**253. Interstate Barriers.** W. H. LIST, Section New York and New Jersey, Ice Cream Manufacturers Assn. *Ice Cream Field* 35: 2, 16, 62, 63. 1940.

Instances are cited in which legislative enactments have resulted in interstate barriers. Special mention is made of a recent law in Connecticut which

requires the pasteurization of ice cream mix within its borders, regardless of where or how it may have been previously pasteurized elsewhere.

The author reproduces several letters from state governors and United States Secretary of Commerce in an attempt to point out the seriousness of the problem. He suggests that legislators be encouraged to correct the condition.

W.C.C.

**254. Storage and Delivery Influence Ice Cream Quality.** W. C. COLE,  
Division of Dairy Industry, University of California. *Ice Cream Field*, 35: 2, 50. 1940.

Improvements in ice cream cabinets and delivery equipment make it much easier to control temperatures during delivery and storage. It is pointed out, however, that proper construction and insulation of the refrigeration compartments of trucks as well as adequate sources of refrigeration at suitable intervals are not sufficient to maintain the desired temperatures unless care is exercised by the operator in eliminating unnecessary opening of refrigeration compartment doors.

The new ice cream cabinets now in use are easily regulated and require relatively little service. The direct expansion type units have largely replaced brine filled units and the cartridge systems formerly employed. He points out that cabinets used for factory filled packages are ordinarily set to operate at temperatures slightly below 0° F.

The author points out the advisability if not the necessity of the use of a checking system which should enable the manufacturer to check the quality of his product on the way from the plant to the ultimate consumer.

W.C.C.

## MILK

**255. A Discussion of Bottle Rinsing Problems.** J. H. HALE. *Can. Dairy and Ice Cream J.*, 19: 2, 52. 1940.

Minimum recognized conditions for producing a satisfactory glass milk bottle are generally given as: (1) soaker solutions of at least 3 per cent alkali strength; (2) caustic content of at least 1.8 per cent; (3) maintained at a temperature of not less than 130° F.; (4) bottle immersion in the solution for not less than 5 minutes. When foam was not prevented the addition of a wetting agent to the soaker solution caused about 50 per cent greater residual alkali in the bottle compared to the non-foaming condition. High temperature of rinse water shows a greater efficiency of rinse. A slightly acidified rinse water was found to remove more residual organisms than a plain water rinse. The rinsing of mixtures of caustic with milder alkalies is for practical purposes, no different from straight caustic. The bacteriological problem is taken care of if the soaker solution is kept up to standard.

O.F.G.

- 256. The Consumer's View-Point.** MRS. G. E. ROBINSON, Can. Dairy and Ice Cream J., 19: 2, 19. 1940.

This article discusses some angles of distributor-consumer relationship which do not seem to have received recognition from many in the fluid milk industry. This consumer suggests that increased milk consumption holds as much interest to the housewife as it does to the producer and distributor.

O.F.G. .

- 257. A Review of Milk Control in Ontario.** C. M. MEEK, Can. Dairy and Ice Cream J., 19: 1, 47. 1940.

This is a review article, which also points to the direction milk control may take under war-time conditions.

O.F.G.

- 258. A Fundamental Factor.** H. B. ELLENBERGER. Can. Dairy and Ice Cream J., 19: 1, 15. 1940.

This article deals with the control of costs of production, assembly, transportation, processing, distribution and consumption. The author concludes that if milk could be produced and sold more cheaply, more would be used; the consumer would be benefited, profits to both the producer and distributor would be more certain and everybody concerned would be better satisfied.

O.F.G.

- 259. Types of Flavours Detected in Milk at the Receiving Platform.** G. M. TROUT. Can. Dairy and Ice Cream J., 18: 10, 41. 1939.

This is a report of a study of flavor the data for which was obtained by sampling 920 cans of milk as they arrived at the receiving platform. Approximately 45 per cent of the cans contained milk which was described as clean and pleasant to the taste. The flavors in the milk of the other 55 per cent of the cans were distributed as follows: feed, 43.19 per cent; musty, 19.92 per cent; high-acid, 14.37 per cent; unclean, 11.44 per cent; barny, 2.56 per cent; cowy, 2.37 per cent; oily, 2.56 per cent; miscellaneous, 3.55 per cent. The author suggests that a change in feeding practices plus adequate cooling would eliminate much of the feed, musty and high-acid flavors which constitute over 75 per cent of the off-flavors present in these milks.

O.F.G.

- 260. The Elwell Plan of Sliding Scale of Prices to Consumers.** EDWIN S. ELWELL, National Milk and Cream Co., Minneapolis, Minn. Milk Dealer, 29: 6, 70-77. March, 1940.

A description of the Elwell plan whereby a sliding scale of prices is applied in the sale of milk and cream is presented. The plan is based on the fact that a certain cost is involved in the delivery of a quart of milk and that

additional quarts delivered at the same stop have a lower delivery cost. The benefit of this lower delivery cost is given to the consumer in the form of a lower price on additional quarts of milk.

C.J.B.

**261. Le Teneur en Matière Grasse du Lait de Femme (The Fat Content of Woman's Milk).** H. GOLTZ, *Le Lait*, 20: 20-29, 145-154. 1940.

The quantity and composition of human milk are known to vary considerably as a result of many conditions. Fat content varies more widely than any other constituent. The literature in this connection is reviewed and the results of the author's studies at the Stuttgart clinic presented.

The relation of period of lactation to fat content indicates that there is a slight increase in fat content as the time advances. Samples taken on the third day *post partum* averaged 2.8 per cent and those from the second to the fourth month 4.2 per cent. There appears to be a slight but characteristic decrease on the tenth day, usually attributable to the resumption of body activity on the part of the mother.

The caloric value of initial milk compared to that of the final milk has been studied. In 85 per cent of the cases the final milk has a higher fat content although the differences in most cases was less than 0.5 per cent.

The results of 285 analyses, grouped on the basis of the age of the mother, indicated that age did not affect fat content. Goltz also found that the quantity of milk produced had little relation to its composition. This finding is contrary to those reported by other workers.

Evidence is presented to show that fever and inflammation markedly affect composition. In almost all cases, high temperatures resulted in an increase in fat content. In cases of serious mastitis the fat content is usually reduced. The age at which sexual maturity is attained also appears to affect the fat content, individuals reaching puberty at an early age tending to display a slightly lower fat content in their milk.

O.R.I.

**262. Comparative Investigations Regarding Goat Milk and Cow Milk.**  
SIGURD FUNDER, *Meieriposten*, 29: 4. Jan. 1940.

Experiments were conducted to determine the correctness or incorrectness of the contention that bacterial development is not the same in goat's milk as in cow's milk, and that cheese from the two milks do not ripen the same.

To minimize the errors that might be caused by differences existing in different individuals, large lots were used and the milk was obtained from different parts of the country and in different seasons. Milk from large and small herds has been used.

A resume of the experiment follows:

1. In general the goat's milk seems to have lower potential and real acidity than cow's milk (lower SH and higher pH). It usually has shorter

reductase time and larger catalase number, as well as larger contents of sediment and foreign substances. The bacterial content is on an average also greater and the fermenting types more undesirable than in cow's milk.

2. In the experiments it can be seen that in a few cases the goat's milk will sour slower than cow's milk but other times faster. There does not seem to be any constant quality in this respect. The milk flora in goat and cow milk varies both quantitatively and qualitatively and is determined by the direction the self inoculation takes.

3. The experiments seem to show that goat's milk contains, to a larger degree, thermolabile material. This can be construed as bactericidal material.

4. By self inoculation the goat's milk regularly reached a higher acidity finally than cow's milk. This can be caused by microbial as well as non-microbial differences in the two milks.

Joel G. Winkjer.

**263. The Use and Value of Special Tests in the Selection of Milk Salesmen.** VERNE STEWARD AND ASSOCIATES. Los Angeles, Calif. The Assn. Bull. Intern. Assn. of Milk Dealers. 32nd year: 11, 251-263. Jan. 1940.

General principles which have been worked out for the selection of men likely to be successful in life insurance selling are applied to the selection of milk salesmen. The "series of hurdles" rating form is used to rate the applicants "unfit," "borderline," "acceptable" or "superior." Several factors are rated separately and weakness in anyone will cause the applicant to rate "unfit." The final hiring decision is, however, a responsibility of management. The Steward Composite Inventory and Examination suggests measures of mental ability; background knowledge; aggressiveness, initiative and leadership; stability; vocational interests. Several other characteristics are to be appraised by direct investigation. It is suggested that the selection of new employees has not received the attention it merits from the association and that a comprehensive field study over a period of months is needed to provide the basic material for a "Manual of Instructions" which would specify selection procedure. The article contained much valuable material with regard to employee selection. E.F.G.

**264. Increasing the Viscosity of Cream.** L. H. BURGWALD, Ohio State Univ. Milk Dealer, 29: (6), 52-54. 1940.

A heat treatment for increasing the viscosity of cream is discussed. This method is to heat cream slowly to 84 to 86° F. in a vat, employing but little agitation. Take about one hour to heat. Then cool slowly (about 3/4 to one hour) to between 60 and 50° F. Draw off the cream in cans and store in ice water until the next day. Then standardize and bottle.

Using this method, an average increase of 8.3, 28, and 52.5 per cent was obtained in the flow time of 20, 22, and 25 per cent cream, respectively, as compared with untreated cream. C.J.B.

**265. California Roadside Improvement Program for Dairy Farms.**

RALPH J. WHEELER, The Assn. Bull. Intern. Assn. of Milk Dealers. 32nd year. No. 8, 202-207. 1940.

An "Award of Merit" is given to a dairy farm which has achieved a score of 90, or above, under rules set up and administered by the California Dairy Industries Association. This movement, originating in the Los Angeles milk shed, has spread to other sections of California. The purpose is to make producing farms so attractive to passers-by that more milk will be consumed. In addition to the metal plaque to be placed on a post in front of the home a certificate suitable for framing is given. Many groups cooperate in fostering this movement. In the current year a dairy organization known as the Society of Yellow Dogs sponsored a Dairy Field Day which netted \$550.00 for the benefit of this work. It is stated that pride taken in improved premises has resulted in many tenants becoming owners, that they might be putting this effort on their own places. E.F.G.

**266. Present Trends in Milk Production in Relation to Future Prices of Dairy Products.** G. E. GORDON, Univ. of Calif. The Assn. Bull. Intern. Assn. of Milk Dealers. 32nd year. No. 8, 196-201. 1940.

Attention is called to the necessity of operating on a high plane of efficiency in both production and distribution. It is suggested that we may have been considering as a normal dairy price level a price relationship in which dairy products have been comparatively high. The current production and consumption prospects are discussed. E.F.G.

**267. The Milk Supply of Large Cities—Discussion of Methods for Safety.** J. C. GEIGER AND B. Q. ENGLE, San Francisco, Calif. The Assoc. Bull. Intern. Assn. of Milk Dealers. 32nd year. No. 8, 187-195. 1940.

San Francisco's fluid milk supply is all of Certified or Grade A quality; is 100 per cent pasteurized and is produced on 190 dairy farms with an average of approximately 100 milking cows per farm. The maximum hauling distance is 131 miles and the average 42. The cream supply is also Grade A quality, produced on 297 farms averaging 60 cows per farm. The per capita consumption of milk is .54 pints and of cream .035 pints. The many sanitary and health requirements surrounding the production and handling of this milk including tuberculosis eradication are outlined. Structure requirements include a barn used exclusively for milking and a two-room milk

house. Milk must be cooled below 45° F. necessitating mechanical refrigeration. The logarithmic average of raw milk counts for 1926-27 was 25,000 colonies per cc. which was gradually reduced to 3,000 in 1938-39. The fat content of the milk has increased from 3.59 to 3.90 per cent during the same period. All milk and cream is brought raw to the 18 city pasteurizing plants. The logarithmic average of pasteurized milk in 1926 was 19,000 while the average for the past 6 years is under 450. Numerous more recent requirements for the market are listed. The author stresses the point that the plate count is a poor index of the milk flora and no index of the safety. The important characteristic of milk from the health officers viewpoint is safety. The answer to safety lies in field control of sanitation, field measures to keep out pathogenic organisms and in pasteurization. E.F.G.

**268. Keeping Records for Determining Bottle Trippage.** ANONYMOUS.  
Milk Dealer, 29: 6, 34, 80. 1940.

A chart is presented, which is used as follows for determining bottle trippage: At the end of each filling period, mark number of bottles filled. At the end of each month put down total bottles bought. At the end of the year, divide the number of bottles bought into the number of bottles filled to find the number of trips per bottle.

C.J.B.

**269. Consumption of Chocolate Milk by Children.** GIDEON HADARY, Univ. of Wis., Madison, Wis. Milk Dealer, 29: 6, 33, 80. 1940.

From a study of the drinking habits of young children the following conclusions were drawn:

1. Children drink less milk as they grow older in spite of the fact that their preference for milk does not decrease.
2. Children shift away from milk drinking because of existing social and cultural influences.
3. Soft beverages are the substitute of the whole milk.
4. Chocolate milk is a beverage, and as such may come to replace the milk.
5. Consumption of chocolate milk does not decrease the total consumption of milk, due to the preference for the drink. The consumption of milk as a whole is increased.

C.J.B.

**270. Stop Sunday Delivery.** ANONYMOUS. Milk Dealer, 29: 6, 31. 1940.

In Akron, Ohio, as a step toward reducing distribution costs, Sunday deliveries have been eliminated. Time of delivery has been changed to begin at 7:30 A. M. instead of 5 A. M. Dealers report that sales have not suffered and that they have received hearty cooperation from the public. A big percentage of the milk sales in Akron is in gallon jugs.

C.J.B.

271. **Cereal Cream.** PAUL VASTERLING, Sanitary Dairy Co., Indianapolis, Ind. Milk Dealer, 29: 6, 30, 44-48. 1940.

The author advocates that cereal cream be used to compete with cream substitutes in order to improve the present low volume of cream sales.

The cereal cream contains from 10 to 12 per cent butterfat and 11 to 13 per cent of solids not fat, the additional solids being obtained from either skim powder or plain condensed. It is homogenized at high pressures, otherwise handled and processed much like ordinary cream. Because of the low fat content, cereal cream is sold under a trade name such as "Breakfast Special," etc.

C.J.B.

272. **General Managerial Plant Problems.** WALTER C. SCHAFER, Borden's Dairy Delivery Co., Los Angeles, Calif. The Assoc. Bull. Intern. Assoc. of Milk Dealers. 32nd year, No. 9, 221-231. January, 1940.

The cost of change over of equipment in terms of labor requirements is calculated as 14 man hours per day when 10 different products were handled in addition to regular milk. The personal-injury frequency rate in all industries is 13.85 per million hours worked, in the food industry 16.79 and in the dairy industry 23.99. It is recommended that ample appropriations be made for carrying out an accident prevention program.

Many items of plant procedure are mentioned which aid in eliminating public health hazard. It is recommended that all prospective employees be given an adequate physical examination before they are finally employed to determine if they are able to meet the physical requirements of the industry and turn out a full day's work without hazard to themselves or fellow workers. The employee should have periodical physical examinations to keep him healthy and efficient. Such health supervision saves the company more than it costs. Several graphs show that more accidents occur on Monday than any other day, that February and September are the high accident months, handling objects causes more accidents than any other cause, that haste and lack of alertness are responsible for larger number of injuries and that of all parts of the body the fingers are most frequently hurt. E.F.G.

273. **Proper Assembly of Fittings and Fabrication of Milk Lines.** E. N. MUZZY, Samark and Co., San Francisco, Calif. The Assoc. Bull. Intern. Assoc. of Milk Dealers. 32nd year, No. 9, 215-220. January, 1940.

The present tendency is away from many crosses and tee fittings and toward simpler layouts, including one piece fittings and the new type threaded sweep ells. Under the direction of the Simplified Practices Committee of the International Association of Milk Dealers equipment design

has been both improved and standardized. The expanded and flared type of fitting has come with stainless steel and eliminated a great deal of poor soldering. An actual plant layout was shown and the piping was installed first in a modern satisfactory manner and then later, showing "horrible" examples of poor type of fittings and improper assembly.

E.F.G.

- 274. A New Method of Preparing Churned Cultured Buttermilk.** GEORGE B. SAUER, Lucerne Cream and Butter Co., Los Angeles, Calif. The Association Bulletin, Intern. Assoc. of Milk Dealers. 32nd year, No. 9, 211-214. January, 1940.

A centrifugal pump is used instead of a butter churn to produce butter granules. The standard procedure for producing churned cultured buttermilk is followed till churning time. In winter it may be necessary to warm the ripened milk to 75 to 76° F. before pumping. A 2-inch pump turning at 1500 r.p.m. is used discharging the culture 10 or 12 inches above the surface or preferably using a concussion chamber to provide a uniform drop. Ten minutes per 100 gallons of buttermilk is usually needed. It is stated that this method produces a slightly thinner body than the regular churn method but this can be controlled by adjusting the per cent of solids not fat. Advantages claimed for the method are lower investment, the whole process carried out in one piece of equipment, better quality because the unsanitary churn is eliminated and also the size of granules can be more easily controlled. A rather complete description of the entire process is given.

E.F.G.

- 275. Contributions à l'Étude du Calcium du Lait. (Contributions to the Study of Calcium in Milk.)** R. VLADESCO. Le Lait, 19: 354. 1939.

The lack of agreement which exists in the older literature among values pertaining to the mineral matter of milk is understandable in the light of modern theories of ionization. Thus the quantity of calcium held by casein as a caseinate is dependent upon the reaction of the medium. In an alkaline medium very little calcium is held in the colloidal caseinate form.

This fact can be taken advantage of in determining calcium in milk without the necessity of drying or ashing the sample. Ten ml. of milk are diluted with about 70 ml. of water and the protein precipitated with copper sulphate and potassium ferrocyanate. After making up to 100 ml. the mixture is filtered and 25 ml. of the filtrate analysed for calcium by the ammonium oxalate-potassium permanganate method.

O.R.I.

- 276. Le Lait et La Vitamine C en Roumanie (Milk and Vitamin C in Rumania).** R. VLADESCO AND MME H. PRAHAVEANU. Le Lait, 19: 798-806. 1939.

The role of vitamin C in physiological oxidations and in the treatment of

diseases of the alimentary tract is discussed. In view of the fact that human infants sometimes have to secure part of their vitamin C from cows' milk, extra care should be exercised to prevent its destruction. Data are presented to show that the cause of variation in the vitamin C content of raw milk is largely atmospheric oxidation. The average value was 16.32 mgm. per litre with a range of from 8.07 to 29.24 mgm. per litre.

O.R.I.

**277. Factors Related to Viscosity Control.** E. WALLENFELDT, Extension Specialist in Dairy Industry, Univ. of Wis., Madison, Wis. Milk Dealer, 29: 5, 70-74, 83-84. Feb. 1940.

The effect of butterfat content, temperature, breed of cow, separating temperature, pasteurizing temperature, aging, cooling time, homogenization, and special temperature treatment, on the viscosity of cream were studied. The following conclusions were drawn:

1. Pasteurization temperature of 143-145° F. gives higher viscosity than higher temperatures with the holder method for 30 minutes.
2. Aging for 24 hours before sale seems desirable from the standpoint of viscosity control.
3. Accurate and uniform fat standardization is important.
4. Cream should be delivered at as low temperatures (above 32) as is practical.
5. Agitation between the temperatures of 90 degrees F. and 40 degrees F. should be as gentle and as uniform as possible.
6. Slower rate of cooling will cause increased viscosity.
7. If local health regulations and plant facilities permit, a viscous cream may be produced by separation of pasteurized milk (at 80-85 degrees F.) which has been held for several hours after pasteurization.
8. Cream of very satisfactory body can be produced by homogenization.

C.J.B.

**278. Thermophilic Bacteria in Pasteurized Milk.** CHARLES C. WALTS. Res. Assoc. Creamery Package Mfg. Co. Milk Plant Monthly, 29: 4, 29. 1940.

Thermophilic "heat loving" bacteria are often responsible for high count "pin point colonies" on plated pasteurized milk. Three laboratory methods; namely, agar plate, direct microscopic and methylene blue and resazurin reductase, have been used to determine whether thermophilic or thermoduric organisms are responsible for high counts and pin point colonies. Control procedures involve quality production procedures on the farm, particularly adequate cooling, and in the plant checking such factors as cleanliness and sterility of cans, deposits of milkstone, cleaning, sterilizing and drying of equipment, repasteurization of milk, long continuous pasteurization, long use of same filter cloth, foam on milk, dead ends of sanitary piping,

allowing milk to remain hot in preheaters and permitting condensation from unsterile surfaces to drop into pasteurized milk.

G.M.T.

**279. Chocolate Milk.** J. G. BRERETON, W. B. COMBS, AND H. MACY,  
Dairy Division, Univ. of Minn., St. Paul, Minn. Milk Dealer, 29:  
5, 38, 62-68. Feb. 1940.

From a study of the factors influencing the physical characteristics of chocolate milk the authors draw the following conclusions:

1. With the exception of one powder and one hot-process syrup, all of the commercial non-settling chocolate milk preparations studied produced a stable chocolate milk when the processing recommendations of the manufacturer were followed.
2. Slow cooling of chocolate milk in the vat will cause cocoa sedimentation if the milk is on the borderline of complete stability as judged when the milk is surface-cooled.
3. Increasing the fat content of the milk used to make non-settling chocolate milk will decrease the amount of cocoa sedimentation when instability results with a milk of a low fat content.
4. With commercial, non-settling preparations, the temperature of the milk at the time of adding the powder or syrup does not affect stability, provided the agitation in the vat is satisfactory and the material does not contain an alginate stabilizer.
5. Increasing the temperature of pasteurization decreases the amount of stabilizer needed to produce a stable chocolate milk.
6. In making non-settling chocolate milk by the cold-process syrup method, adding the syrup to milk as warm as 75 degrees F. does not impair the stability of the chocolate milk and may be considered advantageous because it facilitates the dispersion of the syrup in the milk.
7. Colorless, odorless and practically tasteless stabilizers are available for making chocolate milk.
8. The physical state of certain stabilizers in water suspension is greatly affected by the presence of relatively low concentrations of certain salts.
9. The addition of small amounts of  $\text{CaCl}_2$  to milk causes the gelation of a purified alginate stabilizer in that milk.
10. In general, chocolate milk made with algal stabilizers has a tendency to thicken upon aging at 40 degrees F. as evidenced by the increase in the body flow time.
11. The presence of algal stabilizers in milk can be shown by staining the milk with a dilute aqueous solution of crystal violet. •
12. When chocolate milk is pasteurized at 165 degrees F. for 20 minutes, the minimum amount of the various stabilizers needed for complete stability has been shown to vary from 0.036 per cent up to 0.245 per cent of the weight of the milk used.

13. "Natural" process cocoas could not be used satisfactorily to prepare stable chocolate milk with the alginate types of stabilizer now being sold.

14. When stabilizers are used to prepare chocolate milk, the cocoa fat content and the relative fineness of the average commercial cocoa do not seem to have any appreciable effect upon the stability of the chocolate milk.

C.J.B.

**280. Standardize New Two-Quart Bottle.** ANONYMOUS. Milk Dealer, 29: 5, 37. Feb. 1940.

A joint meeting of the glass container, cap, equipment, and crate industries passed the following resolution:

That a standard two-quart bottle be adopted for the dairy trade with the following specifications:

Height .....	10½ in.
Body Diameter .....	4-25/32 in.
Weight .....	30 oz.

C.J.B.

**281. Towards a Perfect Milk Market.** J. ELIZABETH DONLEY. Mass. Agr. Exp. Sta. Bull. 366.

The bulletin reports a study of the milk supply for the city of Worcester, a secondary milk market in Massachusetts. An analysis of the supply, methods of payment and sales of milk for the year 1935 is made. The conclusion was that an equilibrium of supply and demand, practically speaking, had been established. If any part of the channels then existing should be changed, the amount of surplus would doubtless increase.

The transportation phase seems to be the only part which might be more efficiently organized but that involves personal relationships and should not be regulated by the Massachusetts Milk Control Board.

There is really nothing radically wrong in the market set-up, at least in the supply side of it. The producer sells his milk regularly throughout the year, the dealer has very little surplus to dispose of, and the consumer is assured of a regular supply of good milk throughout the year. H.G.L.

**282. The Control of Sediment in Homogenized Milk.** A. J. HAHN AND P. H. TRACY, Dept. of Dairy Husb., Univ. of Ill., Urbana, Ill. The Dairy World, 18: 10, 28. Mar. 1940.

Homogenized milk held cold ( $40^{\circ}$ - $45^{\circ}$  F.), without excessive agitation, will be free of sedimentation if the cell count is under  $100,000 \pm 25,000$ . If the temperature increases after bottling and if the bottles have been subjected to agitation, then a cell count of 50,000 or less is needed to avoid sedimentation. Clarification is almost essential if the cell count is in excess

of 125,000 in milk to be homogenized. Clarification following homogenization is more efficient than previous to homogenization and multiple clarification is more efficient than single treatment. Destabilization of the milk protein will increase sedimentation and vice versa.

F.J.D.

### MISCELLANEOUS

283. **The Choice of Fuel for a Dairy Plant.** S. KONZO. Can. Dairy and Ice Cream J., 19: 2, 44. 1940.

The author outlines 14 points to be observed in obtaining maximum combustion efficiency of the fuel in use. The choice of the fuel to be used in any given case is a matter to be decided ultimately by the plant owner.

O.F.G.

284. **Some Facts about Merchandising Quick Frozen Foods.** C. Q. SHIERMAN. Can. Dairy and Ice Cream J., 18: 12, 43. 1939.

Quick frozen foods are economical and are better than when fresh. Ice cream manufacturers should be interested in quick frozen foods because they are a "natural" for distribution by the industry. The best type of outlet is the grocer or meat dealer catering to the average wage earning element. Frozen foods must be displayed so as to show how natural they appear. Suggestions as to equipment loans, insurance and margin for the distributor are given.

O.F.G.

285. **Creating Consumer Demand.** F. BEACH. Can. Dairy and Ice Cream J., 18: 10, 15. 1939.

This is a discussion of present day advertising and suggestions are given as to how the best trends in presenting products to the consumer can be used. The author suggests that a judicious combination of research, proper development of customer good will, constant insistence upon cooperation and coordination within the industry, effectively presented by means of an intensive advertising campaign will create a satisfactory consumer demand.

O.F.G.

286. **Nouvelle Application de la Lampe de Quartz à Haute Tension à Vapeur de Mercure Irradiation des Eaux D'alimentation des Chaudières pour Empêcher L'entartrage (New Application of the high tension quartz-mercury-vapour lamp to boiler feed water to prevent scaling).** J. VIEILLY. Le Lait, 20: 142-145 1940.

Water which normally produces a hard, rust-colored scale when evaporated in a boiler, can be treated by irradiation so as to yield a soft type of boiler mud that can be easily removed. Water subjected to this type of

treatment and used for several months will gradually clean up a scaled-up boiler. The author presents no figures as to the type of hardness present in the raw water, however.

The above results are discussed and the possible relationship between irradiated and "Scale-Buoy" treated water pointed out. The question is raised as to whether or not irradiation may not significantly affect the state in which calcium and other minerals are held in milk. It is also suggested that the anti-rachitic potency of such milk may be in part due to the activation or ionization of the calcium.

O.R.I.

**287. Address of the President at the Thirty-Second Annual Convention.**

F. F. RENNIE, JR., Virginia Dairy Co., Richmond, Va. The Assn. Bull. Intern. Assn. of Milk Dealers. 32nd year: 10, 237-248. Jan. 1940.

The growth of the association from 62 members twenty-four years ago when it also met in San Francisco is reviewed. Trends in the industry and activities of the association committees during the past year are summarized. Reference is made to the objectives of the association and measures being taken to attain them. Attention is drawn to the fact that milk sold at lower prices than home delivered milk does not increase consumption, but that higher consumption results from home delivery to the ultimate benefit of the consumer. Recommendations are made with regard to activities which should be pushed during the coming year.

E.F.G.

**288. Pacific Slope Dairying—Before 1850.** ROBERT E. JONES. Pacific Dairy Review, 43: 10. 1939.

The first cattle in many numbers came to California in 1769. At Carmel Mission, milk was practically the only food for eight months, but for the most part there was an immense number of cattle and very little milk. The cattle were valuable for their hides and by 1834 there were over 400,000 of them being raised.

Cattle made their way into Oregon and were divided among the settlers. Jason Lee helped those settlers, who could afford to pay cash, to find ways to get cattle. Ewing Young and several others drove nearly 700 cattle to Oregon. Indians were a menace during the drive.

Development of dairying in Idaho waited until the coming of irrigation. Reverend Colton thought that there was not much possibility for agriculture and dairying in California, but today California stands in second position among all the states in the value of agricultural products.

P.A.D.

**289. Pacific Coast Dairying During the Gold Rush—Article II.** ROBERT E. JONES. Pacific Dairy Review, 43: 12. 1939.

Jacob Harlan was considered the first San Francisco distributor of milk.

In 1850 he bought some cows and employed a man to sell the above mentioned product at four dollars a gallon.

The feeling for improved stock was great in the early fifties; therefore, little regard was given to the preservation of the native breed. Robert Blakon was extensively engaged in the importing and breeding of fine livestock.

The cows driven across the plains were the foundation of dairy efforts, for the growth of dairying after 1850 was rapid. By 1860 the eastern rim of California was well populated by the offspring of the pioneer cows and the production of dairy products was a growing industry. P.A.D.

**290. Modern Methods of Fly Control.** W. A. POHLMAN, Milk Dealer, 29: 6, 40, 64-68. 1940.

A discussion is given of fly control from the standpoint of prophylactic methods and plant openings. The prophylactic methods apply to the territory adjacent to and surrounding the plant property and in the plant proper. The plant openings are discussed from the standpoint of proper closures, erection of barriers, and elimination of unnecessary openings. C.J.B.

**291. Recharting Your Plant for More Efficient Steam Operation.** H. A. MOON. Milk Dealer, 29: 5, 30-32, 57-61. Feb. 1940.

A detailed description of how to chart plant operations. This is followed by instructions for recharting the plant so as to avoid danger points. Examples are given where recharting eliminated the necessity of purchasing a larger boiler. The chart is also applicable for the lighting, power, labor, refrigeration, and water used in any given plant. C.J.B.

**292. Clean Dairy Products.** L. M. DORSEY, Univ. of Maine. Ice Cream Trade J., 36: 3, 53. 1940.

The advantage of water lubricated pumps over oil lubricated pumps for water supplies for food production is pointed out. W.H.M.

**293. Maintaining Plant Efficiency.** H. S. FIELDER, Cherry-Burrell Corp., Chicago, Ill. Ice Cream Field, 35: 2, 20, 22, 74, 75. 1940.

It is pointed out that most people realize the value of proper plant layout, but too frequently they fail to see the need for replacing or remodeling obsolete and worn out equipment.

In the installation of new equipment or the remodeling of a plant it is necessary to check many details which might otherwise fail to be properly handled. Special mention is made of steam and refrigeration.

It is claimed that upkeep and maintenance are fundamental in obtaining good operating efficiency in any plant, but especially in a dairy plant.

Proper maintenance will keep "shut-downs" to a minimum. He advises the proper filing of instructions for maintenance, lubrication, etc., for each piece of equipment which will help prevent difficulties and will help eliminate them if they occur.

May other regularly recognized practices are mentioned in relation to economical plant operation, e.g., noncondensable gases in the refrigeration system raise the head pressure, oil in the evaporators requires lower back pressures at the compressor in order to accomplish the cooling desired.

W.C.C.

## PHYSIOLOGY

294. **The Comparative Anatomy of the Mammary Glands (With special reference to the udder of cattle).** CHARLES W. TURNER, Univ. Cooperative Store, Columbia, Mo. 373 pages, illustrated.

This book is designed primarily as a text for college students of agriculture (dairy and animal husbandry), veterinary science, biology, and medicine. Investigators in these fields will find the book an excellent reference text.

A valuable feature of the book, especially for research workers, is the extensive bibliography following each chapter. For the student the questions with each chapter will prove stimulating in ascertaining his knowledge of the chapter.

The work consists of the following 5 parts: Part I, The Gross Anatomy of the Mammary Glands of Cattle; Part II, Microscopic Anatomy of the Udder of Cattle; Part III, The Comparative Anatomy of the Mammary Gland; Part IV, Anatomy of the Mammary Glands of the Hoofed Mammals (*Ungulata*) ; and, Part V, The Anatomy of the Mammary Glands of the Primates.

This book is well written and admirably illustrated with 54 full-page plates and 14 charts and diagrams. It is unique in having as a source of so much of its material the work of the author.

R.P.R.

295. **Hormonal Inhibition of Lactation.** R. P. REECE, J. W. BARTLETT, I. L. HATHAWAY, AND H. P. DAVIS, Depts. of Dairy Husbandry, N. J. Agr. Exp. Sta. and Nebr. Agr. Exp. Sta. Proc. Soc. Exp. Biol. and M., 43: 183. 1940.

Fifty-six lactating rats were used in this study. An estrogen (Progynon-B) and a gonadotropic principle from pregnant women's urine (Antuitrin-S) were administered separately and also simultaneously. The effectiveness of Progynon-B in inhibiting lactation was increased through the simultaneous administration of Antuitrin-S. Many cells in mitoses were observed in the mammary gland parenchyma of rats so treated. Progynon-B, either alone or with Antuitrin-S, augmented the lactogen content of the pituitary gland.

R.P.R.

**296. Further Evidence for a Mammogenic Factor in the Rat Hypophysis.**

RALPH P. REECE AND SAMUEL L. LEONARD, Dept. of Dairy Husbandry, N. J. Agr. Exp. Sta., and the Bureau of Biol. Res., Rutgers Univ. Proc. Soc. Exp. Biol. and M., 42: 200. 1939.

It was possible to demonstrate an "hypophyseal factor" in rats of both sexes which was capable of inducing growth of the mammary glands in castrated hypophysectomized immature female rats. The administration of estrogens did not influence the "mammogenic factor" when tested in hypophysectomized rats, however, the treatment was sufficient to lower the growth-stimulating power of the hypophyses.

R.P.R.

**297. Lactogen Content of Female Guinea Pig Pituitary.** R. P. REECE,

Dept. of Dairy Husbandry, N. J. Agr. Exp. Sta., Proc. Soc. Exp. Biol. and M., 42: 54. 1939.

The lactogen content of pituitary glands from 48 guinea pigs sacrificed either in estrum, diestrus, early pregnancy, late pregnancy, or the 11th day of the lactation period was determined by injecting the suspended tissue intradermally over the crop gland of common pigeons. The lactogen content was lowest during diestrus and highest during lactation while the glands from guinea pigs in estrum contained more lactogen than did those in early pregnancy and less lactogen than did those in late pregnancy.

R.P.R.

**298. The Respiration of Human Spermatozoa and Their Response to**

**Various Gases and Low Temperatures.** LANDRUM B. SHETTLES,  
Dept. of Obstetrics, Johns Hopkins Univ. and Hospital. Am. J. Physiol., 128: 408-415. 1940.

Carbon dioxide produces complete immobility of human spermatozoa within a few minutes. Motility can be restored if the carbon dioxide is replaced by nitrogen, air, or oxygen as soon as all movement ceases. The toxic effect of the gas is dependent neither upon its acid character nor upon anoxia. Nitrogen, nitrous oxide, and air reduced to a very low pressure do not decrease the motility of the spermatozoa. Helium and pure oxygen increase motility. No data are given relative to the effect of these treatments on the fertilizing ability of the spermatozoa.

D.E.

**299. Die Entwicklung des schwarzunten Niederungsrandes von der Geburt bis zum 5. Lebensjahr und variationsstatistische Untersuchungen einer Population solcher Rinder von der Geburt bis zum 3. Lebensjahr.** ALOYS OTT. Ztschr. f. Tierzücht. u. Züchtungsbiol., 45: 3, 259-308. 1940.

At the University of Breslaw 121 cattle were measured regularly from birth to the age of three years and 34 of these were measured to five years.

A116 ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

Nine measurements besides weight were taken at birth, then monthly during the first year, then quarterly during the second year, then at 2.5 years, and finally at three, four and five years. Measurements are given in absolute numbers, relative to wither height, relative to their initial value, and relative to the values at five years. The discussion includes growth rates, individual and racial differences, and the changing conformation of the animal with age. Standard deviations were computed on the larger group and considerable stress is laid on the coefficient of variation in interpreting growth changes.

J.L.L.

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# JOURNAL OF DAIRY SCIENCE

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## ABSTRACTS OF LITERATURE

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Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

## ABSTRACTS OF LITERATURE

### BACTERIOLOGY

- 300. Microbiology of Paper and Paper-board for Use in the Food Industry.** F. W. TANNER, Univ. of Illinois, Urbana, Ill., E. WHEATON AND C. O. BALL, American Can Co., New York. Amer. J. Pub. Health, 30: 256. 1940.

The authors conclude that paper and paper-board made for use in the food packaging industries is a sanitary product of high order. It is not only made from clean, sanitary, raw materials but also results from a manufacturing procedure in which are several absolutely lethal steps, i.e., cooking, bleaching with chlorine, and hot drying rolls. *Escherichia coli* is not found in paper so made.

M.W.Y.

- 301. Microbiological Content of Paper-board Used in the Packaging of Foods.** J. R. SANBORN, N. Y. State Agr. Exp. Sta., Geneva, N. Y. Amer. J. Pub. Health, 30: 247. 1940.

Very few counts in excess of 500 per gram of disintegrated paper-board were found in 2,877 analyses of milk container board from 13 different mills. Slightly higher counts were found in 1,746 analyses of miscellaneous food package paper and paper-board. Differences in the composition of standard agar and incubation at 30° C. rather than at 37° C. did not greatly affect bacterial counts. Bacteria found were the common saprophytic types.

M.W.Y.

- 302. The Production and Utilization of Lactic Acid by Certain Propionic Acid Bacteria.** A. S. PHELPS, M. J. JOHNSON AND W. H. PETERSON. Univ. Wisconsin, Madison, Wis. Biochem., J., 33: 1606. 1939.

The purpose of the study was to ascertain the conditions under which lactic acid would accumulate and to characterize the lactates formed by some of the propionic acid bacteria. Of the three cultures used, *P. pentosaceum*, *P. technicum* and *P. shermanii*, the first produced only l(+) lactic acid from glucose and arabinose, the second only l(+) lactic acid from glucose. The three organisms failed to racemize optically active lactic acid. The growth rates upon the three optical isomerides varied. The presence of both l(+) and d(−) lactic dehydrogenases in these organisms was demonstrated.

K.G.W.

- 303. Strain Variation in the Root Nodule Organism of White Clover.** W. D. REID. Proc. Soc. Agr. Baet., Abstracts, p. 43, 1939, Aberystwyth, England.

Clover seeds were incubated with six different strains of nodule bacteria.

In field tests only one strain (N.Z.11) showed any significant increase in dry matter and nitrogen content above the untreated control needs.

L.J.Meanwell

- 304. Evaluation of Detergents and Disinfectants.** A. T. R. MATTICK AND E. SHARPE. Proc. Soc. Agr. Bact., Abstracts, p. 38, 1939, Aberystwyth, England.

The bactericidal values of alkaline detergents have been studied and K, N and O values determined for the pure substances and for various mixtures, commercial or compounded. The presence of 0.1 per cent milk was found to decrease considerably the values obtained in its absence.

L.J.Meanwell

- 305. Preservation of Starter Cultures.** A. T. R. MATTICK AND H. J. ROGERS. Proc. Soc. Agr. Bact., Abstracts, p. 40, 1939. Aberystwyth, England.

The centrifuge deposit of a *St. cremoris* culture transferred to broth + 5 per cent serum—dried and frozen under vacuum, retained its vitality after storage at room temperature in the dark for 11 months.

L.J.Meanwell

- 306. A Hippurate Aesculin Broth for the Identification of Str. Agalactiae.** J. G. DAVIS AND H. J. ROGERS. Proc. Soc. Agr. Bact., Abstracts, p. 41, 1939, Aberystwyth, England

Aesculin yeast litmus milk (J. Comp. Path., 52, 132) gives four of the characteristic reactions of *Str. agalactiae*. Attempts to devise a single medium to two of those remaining (viz., hippurate hydrolysis and orange pigment formation with starch) failed. The only modification found to be of any value was the incorporation of aesculin. This medium may therefore be used in conjunction with yeast dextrose litmus milk (J. Dairy Res. 6, 125) to identify *Str. agalactiae* with certainty in eradication schemes.

L.J.Meanwell

- 307. A Homofermentative Tetracoccus Isolated from A.I.V. Silage.** A. CUNNINGHAM AND A. M. SMITH. Proc. Soc. Agr. Bact., Abstracts, p. 9, 1939, Aberystwyth, England.

The organism described produced 3 per cent of lactic acid from dextrose in the presence of calcium carbonate. This is not in agreement with Orla Jensen's statement that "the tetracocci are on the whole very weak acid producers."

L.J.Meanwell

- 308. A Motile Lactobacillus Isolated from A.I.V. Silage.** A. CUNNINGHAM AND A. M. SMITH. Proc. Soc. Agr. Bact., Abstracts, p. 7, 1939, Aberystwyth, England.

So far as is known, this is the first record of motility in a homofermentative lactobacillus.

L.J.Meanwell

- 309. Methods of Reproduction of a Spore-forming Bacillus.** J. C. APLEBY. Proc. Soc. Agr. Bact., Abstracts, p. 11, 1939, Aberystwyth, England.

An aerobic spore former is described that gives rise to coccoid forms which reproduce by budding and the formation of gonidia as well as by fission. Occasionally the cocci revert to the original bacillus.

L.J.Meanwell

- 310. Technique and Uses of a Method of Direct Microscopic Count.** L. A. ALLEN. Proc. Soc. Agr. Bact., Abstracts, p. 13, 1939, Aberystwyth, England.

A modification of the Breed method of counting in which a dropping pipette is used. The technique of staining is described.

L.J.Meanwell

## BUTTER

- 311. Application of the Phosphatase Test to Butter.** E. H. PARFITT, Lafayette, Indiana, W. H. BROWN AND G. W. SHADWICK, JR., Chicago, Ill. Amer. J. Pub. Health, 30: 240. 1940.

No relation existed between the phosphatase value, yeast and mold count, total bacterial count and keeping quality of the butter. Agreement between two laboratories was closer with short methods than with the one hour method. Flash pasteurization yielded a higher percentage of phosphatase positive reactions than vat pasteurization although some plants which were flash pasteurizing consistently yielded negative phosphatase reactions, indicating that the process was not at fault. Under the conditions with which butter is marketed, a significant number of samples will react negatively when fresh, and positively after receiving treatment comparable to the commercial methods of distribution.

M.W.Y.

## CHEESE

- 312. Holes and All: Swiss Cheese Has Historic Background.** MARIE WIDMER. The Pacific Dairy Review, 24: 4, 10. 1940.

In 1291 cheese was treasured as an "essential food." The exportation of it began in the 15th century and with milk and dark bread, formed the staple food of the Swiss people in the 16th century.

Today there are about 2,900 modernly equipped cheese factories in Switzerland. The process of making cheese takes about five or six months; this also includes the fermentation which produces the holes characteristic of Swiss cheese.

Transportation is swift and modern, therefore cheese doesn't suffer from

temperature changes. The variety of cheese is great; the uses, endless. Cheese and butter manufacture in Switzerland absorb 48 per cent of the country's milk production.

P.A.D.

**313. The Volatile Acidity in Relation to the Flora of Cheddar Cheese.**

A. T. R. MATTICK AND E. R. HISCOX. Proc. Soc. Agr. Bact., Abstracts, p. 39, 1939, Aberystwyth, England.

Raw milk cheese show a higher volatile acidity at maturity than heated-milk cheese, this may be due to an early change of flora in raw cheese from streptococci to rod forms. High volatile acid accompanies large numbers of the miscellaneous flora which may stimulate the growth of lactic acid bacteria in raw milk cheese.

L.J.Meanwell

**CHEMISTRY****314. The Determination of Peptide Bonds in Crystalline Lactoglobulin.**

ROLLIN D. HOTCHKISS, Copenhagen, Denmark. J. Biol. Chem., 131: 387. 1939.

The number of peptide bonds in crystalline lactoglobulin has been estimated by determining the increase of amino and carboxyl groups when the protein is completely hydrolyzed by (a) a succession of enzymes, and (b) boiling with mineral acid. The average equivalent of peptide bond corresponds to 115.5 gm. of protein. If a molecular unit of 288 amino acid residues is considered, the total molecular weight is computed to be 33,300, which is significantly different from the figure 39,000 more frequently cited as the molecular weight of lactoglobulin.

K.G.W.

**315. A Fluorometric Method for Determining the Riboflavin Content of Food Stuffs.** A. Z. HODSON AND L. C. NORRIS, Cornell Univ., Ithaca, N. Y. J. Biol. Chem., 131: 621. 1939.

A fluorometric method of determining the riboflavin content of food-stuffs is described. The results of application of the method are in good agreement with micro-biological methods reported by others.

K.G.W.

**316. Electrokinetic Aspects of Surface Chemistry. VI. The Interaction of Gelatin with Casein and Egg Albumin at Surfaces.** L. S. MOYER AND ELSIE Z. MOYER, Univ. of Minn., St. Paul, Minn., and Biological Lab., Cold Spring Harbor, N. Y. J. Biol. Chem., 132: 357. 1940.

"The interaction of gelatin with casein and egg albumin at surfaces was investigated by means of electrophoresis measurements. When particles of collodion are coated with gelatin or egg albumin and then placed in contact with the other protein in the dissolved state, the particles assume a gelatin surface in respect to their electric mobilities. Particles of carbon, quartz,

collodion, or mineral oil placed in a mixture of egg albumin and gelatin likewise became coated with gelatin. These same results were found when the egg albumin solution contained surface-denatured egg albumin and the particles were quartz. In the system, casein-gelatin, over the range between pH 5.8 and 7.8, the resultant surface seems to be determined by the protein which is permitted to coat the particles first. In mixtures of the two proteins to which particles of quartz or collodion were added, the casein seem to diffuse more rapidly to the surface and prevent the adsorption of gelatin for the most part. Particles of casein itself, in the pH range where casein is insoluble, are not influenced much by gelatin except over a pH range which extends from pH 5.2 (slightly above the isoelectric point of gelatin) to 3.7 (a value below the isoelectric point of casein). In this range casein becomes completely coated with gelatin. The nature of the mechanisms and their biological significance is discussed."

K.G.W.

**317. Electrokinetic Aspects of Surface Chemistry. VIII. The Composition of the Surface Film on Fat Droplets in Cream.** LAURENCE S. MOYER, Univ. of Minn., St. Paul, Minn. *J. Biol. Chem.*, **133**: 29. 1940.

An interpretive review is made of previous reports on electrokinetic studies of fat droplets, and surface membranes. An investigation of the surface properties of the fat droplets of cream by means of the microscope method of electrophoresis was made. "Milk fat droplets washed with distilled water and suspended in buffer solutions exhibit electric mobilities which are markedly different from those of casein under the same conditions, with the isoelectric point considerably lower. Although unwashed fat globules were not significantly different in their behavior from washed fat droplets at values above pH 5.8, below this figure the electric mobility curves were markedly different, with the unwashed droplets progressively assuming an electrokinetic behavior more nearly identical to that of casein as the pH was decreased. The isoelectric point of the unwashed droplets was not significantly different from that of casein. It was suggested the behavior of the unwashed droplets is complicated by the presence of casein and that pH values at which casein is very slightly soluble the fat droplet surface becomes contaminated with casein. The isoelectric point of casein is dependent to a certain extent upon the ionic strength in acetate buffers, becoming lower in pH value at higher ionic strength. These data are interpreted to be more closely in accord with the evidence of L. S. Palmer for the existence of a complex of phospholipids and a 'membrane' protein, different from other known milk proteins, composing the fat droplet surface."

K.G.W.

**318. The Nature of Sugar in the Milk and the Carbohydrate Metabolism of Lactating Women.** K. M. DAOUD, Biochemical Dept., Faculty of Medicine, Cairo, Egypt. *Biochem. J.*, **31**: 1. 1940.

In lactating diabetics no glucose is secreted in the milk regardless of the existence or absence of hyperglycemia. The amounts of lactose and other constituents of the milk remain within the range for normal individuals. In diabetics, the active mammary glands, contrary to other glands, prevent glucose leakage, possibly by virtue of their synthetic power for lactose, and are capable of maintaining normal milk composition. K.G.W.

319. **Vitamin in Rat's and in Guinea Pig's Milk.** JAMES HOUSTON AND S. K. KON, Nat. Inst. for Res. in Dairying, Univ. of Reading. Biochem. J., 33: 1655. 1939.

The following values determined by physical and chemical methods for the concentration per 100 ml. of vitamin A, carotene, vitamin B, riboflavin and vitamin C, respectively, in rat's and guinea pig's milks, viz., rat: 0.13 mg.; not measurable, 50-80 I.U.; 0.4-0.8 mg.; 0.4 mg.; guinea pig: 0.09 mg.; 0.011 mg.; 20 I.U.; 0.085 mg.; 29.0 mg.

Compared with cow's milk rat's milk is richer in vitamin B, and riboflavin, but contains much less vitamin C. Guinea pig's milk contains much vitamin C, about half the amount present in average lemon juice. Feeding riboflavin to lactating rats resulted in increased concentration of the factor in milk, but generous administration of vitamin B, or C was without effect. The vitamin B content of rat's milk dropped sharply when a vitamin B deficient ration was used. K.G.W.

320. **Xanthine Oxidase and Milk Flavoprotein.** H. S. CORRAN, J. G. DEWAN, A. H. GORDON AND D. E. GREEN, Biochemical Dept., Cambridge. Biochem. J., 33: 1694. 1939.

A method is described for preparing a flavoprotein which catalyzes the oxidation of hypoxanthine, aldehydes and dihydrocoenzyme I, and is about 1000 times more active per mg. dry weight than milk. The three catalytic activities though associated with the same flavoprotein can be differentially inactivated. The flavin moiety has been shown to be very similar to, if not identical with, flavinadenine nucleotide. K.G.W.

## DISEASE

321. **Bloat in Dairy Cattle.** T. M. OLSEN. S. D. Agr. Exp. Sta. Circ. 27. 1940.

A report on analyses of gases from the rumen of cows which had bloated after consuming various pasture grasses: sweet clover, alfalfa, Sudan grass, sorghum, corn, brome grass and marsh or lowland grass were used in the trials. No striking differences in the kinds of gas found were determinable as between legume and non-legume pastures. CO which is toxic in small quantities in the blood was found to vary in rumen gas, from 0.05 to 0.58 per cent. H<sub>2</sub>S, also toxic in low percentage in the blood, is to be determined

in future analyses. No statements are made relative to probable causes of death by bloating, *i.e.*, whether pressure reduces heart and lung action to the lethal point or whether toxic gases are important as a cause of death.

Author's abstract

### FEEDS AND FEEDING

- 322. Influence of Feeds on Lecithin Content of Milk and Possible Relationship of Lecithin Content to Susceptibility of Milk to Copper-Induced Oxidized Flavor.** I. A. GOULD, W. K. FOX AND G. M. TROUT, Michigan State College, East Lansing, Mich. Food Research, 5: 2, 131. March-April 1940.

Feeds do not influence the lecithin content of milk, which was found to average  $.0461 \pm .0011$  per cent to  $.0474 \pm .0009$  per cent when the cows were fed low and high fat rations respectively. The lecithin content of the milk is fairly constant regardless of variations in the percentage of fat; thus, as the per cent of fat in milk increases, the per cent of lecithin in the fatty extract decreases. There is no relationship between the amount of lecithin in milk and its susceptibility to copper-induced oxidized flavor.

F.J.D.

- 323. Feeding the Dairy Herd.** W. B. NEVENS. Ill. Agr. Exp. Sta. Circ. 502: 1-32. Feb. 1940.

A practical brief manual of feeding for dairy farmers. Among the topics included are : (1) qualities of a good dairy ration, (2) classification of feeds, (3) barn feeding, (4) grain mixtures, (5) pasture feeding, (6) method of calculating mixtures, (7) special care at calving time, (8) feeding young stock, (9) special problems in dairy cattle feeding, (10) feeding value of individual feeds. Tables showing feed value yielded per acre by various Illinois crops and nutrients in 100 pounds of various dairy feeds are given.

O.R.O.

- 324. The Preservation of the Grass Juice Factor in Silage.** B. C. JOHN-  
SON, C. A. ELVEHJEM, W. H. PETERSON AND H. J. FAGEN, Univ.  
of Wisconsin, Madison, Wis. J. Nutrition, 18: 527-35. 1939.

The "grass factor" which is found in milk from cows on pasture is preserved in forage by ensiling. The A. I. V. and phosphoric acid methods of ensiling gave better preservations of the "grass juice factor" than did molasses. Milk from cows fed phosphoric acid alfalfa silage was found to be approximately as rich in the "grass juice factor" as milk produced by cows on summer pasture.

C.F.H.

### FOOD VALUE OF DAIRY PRODUCTS

- 325. Comparison of Nutritive Value of Refined Coconut Oil and Butterfat.** R. S. HARRIS AND L. M. MOSHER, Massachusetts Institute of

Technology, Cambridge, Mass. Food Research, 5: 2, 177. March-April 1940.

In apparently well controlled experiments, rats were fed complete fat-free diets supplemented, in one group, with refined coconut oil, and in another, with pure butterfat. A control group received a standard stock ration. The experimental diets were normal in all respects except that they contained an abnormally large proportion of fat. The animals fed coconut oil increased in weight more rapidly than those fed butterfat, but the increase was not due to adipose tissue. Animals on both experimental diets developed slight fatty infiltration of the body and liver cytoplasm. No evidence was found of pathological tissue changes in any of the animals. The results indicate, according to the authors, that butterfat and coconut oil, even when fed at rather high levels in a complete diet, are equally harmless to rats and presumably to man.

F.J.D.

**326. Influence of Sunlight on Flavor and Ascorbic Acid Content of Milk Exposed in Three Different Types of Paper Containers.** J. L. HENDERSON, D. C. FOORD AND C. L. ROADHOUSE, University of California, Davis, Calif. Food Research, 5: 2, 153. March-April 1940.

Three types of paper milk containers of different paper stocks were compared for protective effects against flavor production and ascorbic acid destruction caused by exposure to sunlight. All three of the paper containers exhibited greater protection than did the clear glass milk bottle, but there was considerable variation in the degree of protection afforded by the paper containers. The container providing the greatest protection was one made of thick paper with unbleached or colored inner plies. The destruction of ascorbic acid by sunlight was found to be a useful index of the effect of the sunlight on the flavor of the milk.

F.J.D.

**327. The Importance of Economical Milk in Human Nutrition.** MARIETTA EICHELBERGER, Chicago, Ill. Amer. J. Pub. Health, 30: 169. 1940.

Summaries of reports concerning the place of evaporated milk in human nutrition are presented. Evaporated milk is popular for infant feeding because of its curd character and easy digestibility. It is a satisfactory source of calcium, phosphorus and nitrogen. Evaporated milk is an important source of vitamins. About 50 per cent of the country's evaporated milk supply is irradiated and about 10 per cent is reinforced with a vitamin concentrate. The need is vital in human nutrition for an economical milk supply.

M.W.Y.

**328. The Food Value and Economics of Skim Milk.** J. S. ABBOTT, Washington, D. C. Amer. J. Pub. Health, 30: 237. 1940.

Facts are given about the food value of skim milk. Ninety per cent of the food nutrients or food value of skim milk is wasted when it is fed to pigs for conversion into pork. The writer states that there ought not to be any restrictions against compounding skim milk with other foodstuffs for use as human food.

M.W.Y.

**329. Whey as a Source of Vitamins and Vitamin Products.** G. C. SUPPLEE, Bainbridge, N. Y. *Ind. Eng. Chem.*, 32: 238. 1940.

Whey, or a concentrated fraction thereof, has been shown to contain lactoflavin (riboflavin, vitamin B<sub>2</sub> or G), thiamine (vitamin B<sub>1</sub>), the anti-aerodinia factor (vitamin B<sub>6</sub>), prothrombin (vitamin K), provitamin D, the oestrogenic hormone, and by permissible deduction, vitamin E and factor W. The multiplicity of vitamins found in the whey fraction after removal of protein and milk sugar is adequate in water-soluble factors to support growth, reproduction, and lactation. Eight successive generations of white rats have been maintained with a normal life cycle on a restricted experimental diet in which the whey vitamin fraction supplemented with rice polish served as the sole source of all vitamins except the fat-soluble factors carried by a small percentage of cod liver oil.

B.H.W.

**330. The Vitamin A Content of Cheese.** A. W. DAVIES AND THOMAS MOORE, Univ. Cambridge and Medical Research Council. *Biochem. J.*, 33: 1645. 1939.

English cheddar cheese was found in biological tests to have a vitamin A potency of an order (7.5 I.U. per gram) that would be expected from its milk fat content, and from the result of colorimetric estimations of vitamin A and carotene. Colorimetric determinations on other full milk cheese gave similar results. Lower values were found for cheeses of lower fat content.

K.G.W.

**331. Adequacy of a Milk Diet for the Rat.** L. R. RICHARDSON AND A. G. HOGAN, Univ. of Missouri, Columbia, Mo. *J. Nutrition*, 19: 13-19. 1940.

The addition of iron, copper, manganese and iodine to a milk diet of rats resulted in the production and weaning of almost as many young per litter as did the controls. The experimental animals produced about half as many litters per female as did the controls. The authors concluded that apparently milk is deficient in some nutrient other than iron, copper, manganese or iodine, which is essential for normal reproduction.

C.F.H.

**332. The Determination of Ascorbic Acid in Commercial Milks.** W. W. WOESSNER, C. A. ELVEHJEM AND H. A. SCHUETTS, Univ. of Wisconsin, Madison, Wise. *J. Nutrition*, 18: 619-626. 1939.

The authors described a method for determining the ascorbic and dehydro ascorbic acid in raw and commercially pasteurized milk. High pro-

ducing cows do not necessarily give less ascorbic acid per liter than low producers. Milk from the Brown Swiss herd contained the most ascorbic acid per liter followed by Jerseys, Guernseys, and Holsteins respectively.

Samples of raw, certified, certified Guernsey and certified vitamin D milks were only a little below fresh milks indicating but a small loss of ascorbic acid from the cow to the consumer. Commercial pasteurized milk contained about half as much ascorbic acid as fresh raw milk and significantly less ascorbic acid than commercial unpasteurized milks. Mineral modification and homogenization apparently have a destructive effect on ascorbic acid.

C.F.H.

- 333. Why Your Customers Need More Dairy Products.** NINA SIMMONDS, Univ. of Calif., San Francisco, Calif. Assn. Bull., Int. Ass. Milk Dealers, 32nd year: 333-338. Feb. 1940.

Emphasis is placed upon the value of milk from standpoint of protein, calcium and phosphorous. Menus are given. For each menu is given the content of the several food constituents and calorific values.

E.F.G.

- 334. The Vitamin A Content of "Light White" Casein.** M. K. MAITRA AND THOMAS MOORE. Nutr. Lab., Univ. of Cambridge, and Med. Res. Council. Biochem. J., 33: 1648. 1939.

"Light White" casein contained enough vitamin A to promote slow growth in rats depleted of the vitamin when included in the diet at the level of 20 per cent. Hot alcohol extracts of the casein were colored yellow and gave blue colorations when treated with  $SbCl_3$  reagent. The value of one I.U. per gram may be taken as the approximate vitamin A content of typical casein. Ether and ethylene dichloride, used in the cold, extracted only a small fraction of the fat present. The vitamin A activity of the casein was at least partially retained.

K.G.W.

## ICE CREAM

- 335. Factors Affecting Viscosity and Coverage Value of Chocolate Coatings for Ice Cream.** HAROLD COLLINS AND J. H. ERB, Ohio State Univ., Columbus, Ohio. Ice Cream Rev., 23: 1, 28. 1939.

The incorporation of small amount of moisture in chocolate coating for ice cream bars increases the viscosity of the coating and decreases coverage value. Coating to which lecithin was added in amounts up to 0.4 per cent resisted moisture thickening. Results obtained under commercial plant conditions showed that when this amount of lecithin was added, the number of bars coated per pound of chocolate was increased.

J.H.E.

- 336. Ice Cream Cabinet Maintenance.** ANONYMOUS. Ice Cream Rev., 23: 1, 42. 1939.

Moisture in a refrigerating system using F-12 as a refrigerant will freeze at the expansion valve orifice causing it to stick on either the open or closed

position. At this sign of trouble the entire system should be checked for the leak and repaired. Then a suitable chemical dryer should be used to dehydrate the system. Several satisfactory dryers are mentioned.

J.H.E.

**337. New Sweetening Agents for Ice Cream.** P. H. TRACY, Univ. of Ill., Urbana, Ill. *Ice Cream Rev.*, 23: 6, 35. 1940.

When the manufacturer replaces sucrose in ice cream with substitute sweetening agents, he should expect differences in results. The type of sweetness may not be the same, but this does not necessarily mean a disadvantage. All monosaccharides will depress the freezing point more than will cane or beet sugar, but when they are used in limited amounts this is not a serious problem. The lower freezing point of mixes containing monosaccharides may cause some ice creams (especially high-fat products) to be more palatable and have a more desirable body. Some sweetening agents such as "sweetose" may contain substances that result in a better body in the ice cream; others such as honey may add desirable flavoring constituents. In the selection of the nonsucrose sweetening agent, the manufacturer should be governed by cost as well as by the effect of the sugar upon the flavor and physical characteristics of the ice cream.

J.H.E.

**338. Stepping up Sales Despite Price Competition.** ANONYMOUS. *Ice Cream Rev.*, 23: 3, 34. 1939.

Eliminating all mention of price and avoiding controversial discussion, the Arctic Ice Cream Company, of Kansas City, Missouri, invaded the cut-price market and increased sales by following an advertising plan consisting of three elements: (1) the development of a trade mark in the form of a stylized polar bear cub, which was easily recognizable and which had unusual attention value, (2) the constant repetition of one idea, associating Arctic ice cream with quality, and (3) the promotion of a merchandising idea which contained appetite and flavor appeal. Details of the campaign are described.

J.H.E.

**339. Merchandising Quick Frozen Foods.** CHARLES Q. SHERMAN. *Ice Cream Rev.*, 23: 4, 40. 1939.

Reasons are cited why ice cream manufacturers should be interested in merchandising quick frozen foods. Principles of merchandising these foods are given.

J.H.E.

**340. Pineapple Ice Cream.** C. D. DAHLE AND D. V. JOSEPHSON, Pennsylvania State College, State College, Pa. *Ice Cream Field*, 35: 4, 24, 44, 45, 48, 49. April 1940.

The results of experiments with pineapple as sources of flavor for ice cream are reported. It was found that best results were obtained when a mixture of unsweetened canned pineapple and 25 per cent sugar was used,

allowing this mixture to stand sufficient time for the sugar to penetrate the fruit before being added to the ice cream. This required 7 hours at room temperature or 12 to 14 hours at 34° F.

It is reported that when ice cream is frozen in a continuous freezer using a fruit feeder, it is best to add the juice to the mix before freezing. Because of improved appearance a new cubed pineapple, especially developed for the ice cream industry, was superior to crushed or tidbit pineapple products. In the case of batch frozen ice cream, crushed pineapple proved as satisfactory as cubed pineapple.

High copper contents in certain pineapple products contributed towards the development of oxidized flavor in ice cream. It is possible to obtain canned pineapple, however, low enough in copper so as not to cause this difficulty.

The authors recommend using 15 to 20 per cent unsweetened or natural pineapple, properly sugared, for flavoring ice cream and give several recipes for batch and continuous freezers.

W.C.C.

**341. Servicing Ice Cream Cabinets.** W. E. WEAVER, Borden Assd. Companies, Toronto, Canada. *Refrig. Eng.*, 39: 5, following 336. May 1940.

This is a condensed treatment of ice cream cabinet servicing requirements. Careful checking of units at intervals cuts down on failure when cabinets are handling seasonal peak loads. The installation of two compressors, one to handle the ice cream cabinet temperature, and the other the back bar, salad pan, sweet water cooling, etc., at 35° to 40° F. in combination outfits is recommended. The proper use of driers will materially lower service calls. Preventive service pays dividends.

L.M.D.

**342. Sanitation at the Soda Fountain.** J. L. RICE, Com. of Health, New York City. *Ice Cream Field*, 35: 4, 19. April 1940.

It is claimed that personal hygiene is one of the greatest problems in connection with public health law enforcement. The author states that all fountain employees should adhere to these rules. (1) All workers must be clean in person at all times, (2) all employees should wear clean uniforms and caps, (3) fingernails and hands should be washed at regular intervals, (4) hair should be neatly combed and a cap worn at all times, (5) hands should be washed after using toilet, (6) periodic health examinations should be required.

The author advocates advertising sanitation and cleanliness, claiming that fountain operators can profitably publicize their employee health rules in order to familiarize the consumers with the high standards maintained.

W.C.C.

**343. Progress in the Ice Cream Industry.** R. A. BRODESSER, Southern Dairies, Inc., Washington, D. C. *Refrig. Eng.*, 39: 6, 377. June 1940.

Refrigeration is the basic factor in the ice cream industry requiring the largest individual investment in machinery. The use of pre-coolers and headers together with booster compressors makes for flexibility in temperatures and efficiency of operation. Bin type direct expansion coil arrangement in hardening rooms is most satisfactory.

Truck body refrigeration should not be expected to act as a hardening agent in addition to transportation protection. Truck temperature should not be allowed to rise above 10° F. This temperature maintenance may be obtained by a hold-over system, truck-operated compressor, or a CO<sub>2</sub> circulating system.

The author points out the advantages of the sealed non-toxic gas compressor for ice cream cabinet use. It is predicted that in areas where the cost of CO<sub>2</sub> equals that of purchased power, electrically operated compressors will become obsolete.

L.M.D.

## MILK

- 344. The Use and Future of Two-Quart and Gallon Milk Bottles.** SCOTT FARON, Glass Container Assn. of America. *Milk Dealer*, 29: 7, 96-109. April 1940.

The advantages of the two-quart container over the gallon container are discussed. The reaction of milk dealers to these containers is also given.

C.J.B.

- 345. The Milk Dealer's Duty in Stabilizing His Industry.** C. W. HOLDAWAY, V. P. I. Dairy Dept., Blacksburg, Va. *Milk Dealer*, 29: 7, 54-56. April 1940.

The author points out that the relations between dealers and producers are as important as the relations between dealers and consumers in stabilizing the milk industry. Several methods are suggested for building producer confidence and developing producer services.

C.J.B.

- 346. State Requirements on Sediment Testing of Milk and Cream.** ANONYMOUS. *Milk Dealer*, 29: 7, 42-44. April 1940.

A tabulation showing the sediment testing requirements and the extent to which they are enforced in 45 states and the District of Columbia.

C.J.B.

- 347. What Do We Know about Consumption of Milk by Consumers.** EDWARD FISHER BROWN, Milk Research Council, New York City. *Milk Dealer*, 29: 7, 37, 72-76. April 1940.

A report of a study to determine the milk-drinking habits of adolescents and adults.

C.J.B.

- 348. Processing and Sale of Homogenized Milk.** ANONYMOUS. *Milk Dealer*, 29: 7, 32. April 1940.

A survey by the Milk Dealer in which replies were received from 603 dealers in 46 states and the District of Columbia showed that 216 of these dealers now produce and distribute homogenized milk, and 77 declared that they expect to homogenize milk for bottling purposes some time in the future.

A homogenizing pressure of 2500 pounds to 2999 pounds was used by 113 dealers, and 168 advised that they used a pressure between 2000 and 3000 pounds. One reported a pressure under 500 pounds and 14, under 1000 pounds.

The report on clarification showed that 110 dealers do not clarify, 72 clarify after homogenization and 28 clarify before homogenizing.

The survey also showed that 98 dealers charged the same price as that for unhomogenized milk, 111 received a premium of one cent, and 6 received a premium of 2 cents a quart for homogenized milk. C.J.B.

**349. "Oxidase Reaction" of Bacteria in Relation to Dairy Products.**

C. H. CASTELL AND E. H. GARRARD, Ontario Agr. College, Guelph, Ont. Food Research, 5: 2, 215. March-April 1940.

An oxidase test is described which is advocated as a means of detecting organisms which may be responsible for oxidative spoilage of dairy products. The oxidase activity of a number of different, common organisms is presented and a discussion of the practical application of the oxidase test as a regular diagnostic procedure is included. The authors believe that interest in the non-living catalysts, which cause oxidation in dairy products, has eclipsed to too great a degree the study of microbial action in this connection. F.J.D.

**350. More about Modernizing the Milk Bottle.** ANONYMOUS. The Pacific Dairy Review, 24: 4, 16. 1940.

One of the latest milk bottles is the two-quart size container which is less bulky to use, takes less space in the refrigerator, and is more in line with the family needs. It packs in cases which stand in one quart spaces and makes economical use of truck space. The gallon container may also be used, but the half-gallon is preferable in dairy plants. The two-quart bottle has increased the volume.

The latest development is a new shaped one-quart bottle constructed of tough glass weighing only 17 ounces, instead of the present 22 ounces, and is designed to effect space economy in the ice box. P.A.D.

**351. Needle Puncture Method for Determination of the Bacterial Contamination of Paraffined Milk Containers.** C. S. MUDGE AND D. C. FOORD, Univ. of Calif, Davis, Calif. Amer. J. Pub. Health, 30: 273. 1940.

Due to the high incidence of sterile paper milk containers, plating meth-

ods for determining bacterial contamination are difficult to control. The needle puncture method prevents outside contamination. Sterile broth is introduced through the wall of the container, the inoculation being made through a drop of alcohol. After allowing from 30 to 40 cc. of media to flow into the container, the needle is withdrawn, and the hole is sealed with hot, sterile paraffin. The container is incubated for 48 hours at 37° C., after which it is opened and an observation made as to the presence or absence of growth.

M.W.Y.

**352. Recent Investigations of Goat's Milk.** A. K. BESLEY, Beltsville, Maryland. Amer. J. Pub. Health, 30: 182. 1940.

Milk from the Toggenburg and Saanen breeds of goats was found to be a healthful, nutritious food, not unlike the milk from the Holstein breed of cows in general composition and nutritive value. The goat's milk studied had a curd 31 per cent softer than that of the milk from Holstein cows and a small-sized fat globule of one-half the volume. Data are presented to show that it should not be difficult for goat milk producers to meet the usual requirements of public health officials for the production of milk.

M.W.Y.

**353. Accuracy of Plate Counts Made from Milk Products as Affected by the Temperature of Incubation.** CARL S. PEDERSON AND ROBERT S. BREED, N. Y. Agr. Exp. Sta., Geneva, N. Y. Amer. J. Pub. Health, 30: 162. 1940.

Studies on the temperature of incubation and the various types of incubators are discussed. A variation of  $\pm 1^{\circ}$  C. in incubating temperature at 32° C. causes on an average only a 4 per cent variation in count, while a variation of  $\pm 1^{\circ}$  C. at 37° C. causes an average error of more than 25 per cent. Many of the present unsatisfactory types of incubators would be satisfactory for milk control laboratories if the present 37° C. temperature were changed to 32° C. Counts obtained at 32° C. are a more constant percentage of the total maximum counts obtainable than are counts at 37° C.

M.W.Y.

**354. Resazurin and Methylene Blue as Indicators of the Hygienic Quality of Raw Milk.** S. B. THOMAS, B. F. THOMAS, AND J. DAVIES. Proc. Soc. Agr. Bact., Abstracts, p. 31, 1939, Aberystwyth, England.

The reduction of resazurin to purple-pink takes about half the time required for complete reduction of methylene blue. Mastitis samples are detected more frequently by resazurin.

L.J.Meanwell

**355. Pasteurized Milk. I. Preliminary Results of a Survey of Plants in the Midlands.** A. L. PROVEN AND A. ROWLANDS. Proc. Soc. Agr. Bact., Abstracts, p. 15, 1939, Aberystwyth, England.

Unsatisfactory pasteurization was found to be due to: (a) inaccurate thermograph readings, (b) hand control of steam valves, (c) common pipe lines for raw and pasteurized milk, (d) deliberate under pasteurization.

L.J.Meanwell

**356. Pasteurized Milk. II. Raw Milk as a Source of Thermoduric Organisms.** A. L. PROVEN AND A. ROWLANDS. Proc. Soc. Agr. Bact., Abstracts, p. 19, 1939, Aberystwyth, England.

High pasteurized counts were traced to unsatisfactory raw supplies due to neglect in cleaning and sterilization of farm utensils.

L.J.Meanwell

**357. Pasteurized Milk. III. The Relationship Between the Colony Count and Various Temperatures and Keeping Quality.** A. L. PROVEN AND A. ROWLANDS. Proc. Soc. Agr. Bact., Abstracts, p. 23, 1939, Aberystwyth, England.

No indication of the keeping quality of pasteurized milk was obtained from colony count.

L.J.Meanwell

**358. Present Developments in the Phosphatase Test.** F. K. NEAVE. Proc. Soc. Agr. Bact., Abstracts, p. 29, 1939, Aberystwyth, England.

A one hour rapid test has been evolved that is as sensitive as the 24 hour test.

L.J.Meanwell

**359. The Pasteurization of Skim Milk and Whey by Direct Steam Injection.** A. T. R. MATTICK, AND W. A. HOW. Proc. Soc. Agr. Bact., Abstracts, p. 27, 1939, Aberystwyth, England.

Steam is injected after the milk leaves the regenerative section of the heat exchanger. Treatment for 3 seconds at 170° F. resulted in the destruction of *B. Coli* and *M. tuberculosis*.

L.J.Meanwell

**360. Observations on Resazurin and Methylene Blue Tests.** J. G. DAVIS AND C. C. THIEL. Proc. Soc. Agr. Bact., Abstracts, p. 35, 1939, Aberystwyth, England.

Resazurin tests with readings at  $\frac{1}{2}$  and 2 hours without inversion give information of great practical value.

L.J.Meanwell

**361. Trials with Insulating Material for Use with Milk Samples During Transit.** H. BARKWORTH. Proc. Soc. Agr. Bact., Abstracts, p. 37, 1939, Aberystwyth, England.

Expanded rubber containers were found to give the most satisfactory protection against an early rise in temperature of milk samples.

L.J.Meanwell

- 362. Selling Extra and Premium Products on Retail Routes.** HAROLD S. WAKEFIELD, Adohr Milk Farms, Los Angeles, Calif. The Assn. Bull., Int. Assn. Milk Dealers, 32nd year: 317-322. Feb. 1940.

The importance—even necessity—of a considerable portion of route business in the form of premium and extra products, if the route is to be profitable, is emphasized. The author states that over half the sales of the company he represents is in the form of these products. E.F.G.

- 363. Building Fresh Milk Volume through Cooperative Promotions.** C. F. DEYSENROTH, The Milk Foundation, Chicago, Ill. The Assn. Bull., Int. Assn. Milk Dealers, 32nd year: 322-327. Feb. 1940.

The lines along which the Pure Milk Association of the Chicago area and the Milk Dealers Bottle Exchange cooperated in promoting the use of milk are explained. The slogans and other material used in the approach to adults are given. The program was directed toward teachers, civic groups and the home. E.F.G.

- 364. Increasing Sales through Industry Advertising.** JOHN MARSHALL, Jr., Milk Dealers Assn., San Francisco, Calif. The Assn. Bull., Int. Assn. Milk Dealers, 32nd year: 327-332. Feb. 1940.

Following a decrease in the per capita consumption of milk in the Los Angeles area, a survey showed that 60 per cent of the adults of the city did not drink milk. Cooperative industry advertising representing 80 per cent of the milk sold resulted in definite improvement in the situation.

E.F.G.

- 365. Relation of Bacteria and of Oxygen to the Flavor of Milk Susceptible to Becoming Oxidized.** J. G. LEEDER AND E. O. HERRIED. Vt. Agr. Exp. Sta. Bull. 457. 1940.

Cows known to produce milk susceptible to becoming oxidized within a short period were machine milked and, without releasing the vacuum in the pail, samples were immediately drawn by vacuum into flasks. Portions were stored under partial vacuum and under atmospheric conditions. The milks were judged for flavor.

Evacuation and holding milks under reduced pressure of 24-25 inches of mercury usually inhibited the occurrence of oxidized flavors during storage periods of 48 hours at 40° F. The growth of bacteria, normally present or added, decreased the oxygen content of the milks and, in most cases, lessened the intensity of the oxidized flavor. J.M.F.

- 366. The Dissolved Gases in Milk and Dye Reduction.\*** J. M. FRAYER. Vt. Agr. Exp. Sta. Bull. 461. 1940.

A quantitative study was made of the dissolved gases in milk as they might be affected by sunlight, metabolic activity, evacuation and processing.

Evidence is submitted that: milk is not oxygen free as it exists in the udder; bacterial increases are accompanied by oxygen exhaustion and reduction time decreases; the point at which methylene blue fading is first noted coincides closely with reduction of resazurin to a full pink and occurs at an oxygen level usually less than 0.045 percentage volume which is reached a variable length of time before reduction; a sharp upturn in carbon dioxide content and a definite increase in residual gas content occurs during this period; in milk subjected to sunlight, oxygen is rapidly dissipated, the presence of dye increases initial speed, bacterial growth is inhibited, reduction times are not decreased when oxygen is first depleted and dye in milk can be reduced without evidence of metabolic activity; and that greatly decreased reduction times may be attributable to depletion of all gases by evacuation but augmentation of oxygen content does not of necessity materially lengthen them.

The quantitative nature of gaseous rearrangements incidental to the pasteurization, cooling, and handling of milk were studied.

Reduction of either dye may occur in two steps: (1) Oxygen exhaustion to a minimum level; and, (2) Mobilization and transference of hydrogen to the dye molecule.

Author's abstract

**367. Refrigeration in Milk Plants.** Application Data 13. E. H. PAULSEN, Fairfield Western Maryland Dairy, Baltimore, Md. Refrig. Eng., 39: 5, 293. May 1940.

Applications of modern refrigeration practices in connection with country handling of milk and the cooling and storage of milk in city plants are described. Some discussion is devoted to by-product refrigeration demands. Items to be considered in milk plant design to achieve the most efficient usage of mechanical refrigeration equipment are stressed.

L.M.D.

## PHYSIOLOGY

**368. Activity of Progesterone in Spayed Females not Pretreated with Estrin.** HANS SELYE, McGill Univ. Proc. Soc. Exp. Biol. and Med., 43: 343. 1940.

Experiments showed that the daily administration of 15 mg. of progesterone to spayed rats produced progestational changes in the endometrium, vaginal mucification, and mammary gland development similar to that seen in late pregnancy. It was concluded that progesterone can exert all its characteristic actions in spayed rats without sensitization by estrogens.

R.P.R.

**369. Studies on the Bovine Electrocardiogram. I. Electrocardiographic Changes in Calves on Low Potassium Rations.** J. F. SYKES AND B. V. ALFREDSON, Michigan State College. Proc. Soc. Exp. Biol. and Med., 43: 575. 1940.

Four calves were placed on a semipurified ration which analyzed 0.10-0.12 per cent potassium. Four control calves received the same ration except that potassium was added to bring the level to 0.35 per cent. Calves were placed on this ration at 160 days of age. Electrocardiograms were usually taken at monthly intervals, serum potassium determinations were made on all calves at 2-week intervals, and plasma calcium, phosphorus, and magnesium determinations were carried out at weekly intervals. The lowest serum potassium level of 4 experimental animals was (in mg. per cent) 10.2, 11.3, 10.7, and 13.8. The serum potassium values for the 4 control calves averaged respectively (in mg. per cent) 21.4, 20.4, 20.5, and 21.6. The calcium, phosphorus, and magnesium plasma values remained within normal limits in both groups. Three of 4 experimental animals showed pronounced changes in the electrocardiogram, the outstanding change consisted in a pronounced increase in the duration of the QRS.

R.P.R.

**370. Studies on Bovine Electrocardiogram. II. Bundle Branch Block.**

B. V. ALFREDSON AND J. F. SYKES, Michigan State College. *Proc. Soc. Exp. Biol. and Med.*, 43: 580. 1940.

Branches of the His-bundle were sectioned in a group of calves and dogs. Electrocardiograms were obtained before and after section. In 6 instances the presence of complete bundle branch block was proved by the onset of complete A-V block and bundle branch block was successfully produced in 14 calves, and in 10 dogs. Changes in the duration and form of QRS after section of the branches of the His-bundle were much less pronounced in calves than in dogs. The difference was attributed to differences in the distribution of the intraventricular conducting system.

R.P.R.

**371. A Comparison of Methods of Assay of the Lactogenic Hormone.**

A. G. BERGMAN, J. MEITES AND C. W. TURNER, Univ. of Missouri. *Endocrinology*, 26: 716. 1940.

Three methods of assay of the lactogenic hormone, of increasing sensitivity, depending upon the proliferation of the crop gland of the common pigeon were compared. Using the McShan-Turner pigeon unit as a standard, it was shown that the Reece-Turner method required only 4.5 per cent as much hormone and the minimum micro-unit only 0.56 per cent as much hormone to produce a unit response. In comparison with the pigeon methods, 272 McShan-Turner units were needed to induce copious lactation in pseudo-pregnant rabbits. Evidence was presented which indicates that the AP factor which stimulates pigeon crop gland proliferation is identical with the factor which initiates lactation in mammals.

R.P.R.

**372. The Relation of the Anterior Pituitary to Bile Production.** O. B.

HOUCHEIN AND C. W. TURNER, Univ. of Missouri. *Endocrinology*, 26: 821. 1940.

The average hourly secretion of bile by 7 female guinea pigs weighing between 280 and 370 gm., taken at a definite time of the day under avertin and ether anesthesia, was found to be 2.94 cc. with a range of 2.5 to 3.2 cc. Following the injection of 25 mg. of an initial extract of the anterior pituitary, there was a rise in bile secretion extending from 4 to 8 hours. The average hourly production of bile from 13 injected animals was 5.45 cc., an increase of about 85 per cent.

R.P.R.

**373. Growth of Vitamin Deficient Rats Treated with Thymocrescin.**

ALBERT SEGALOFF AND WARREN O. NELSON, Wayne Univ. Endocrinology, 26: 860. 1940.

A purified aqueous extract, thymocrescin, was prepared from calves' thymus glands. The preparation agreed closely with published chemical analyses. No growth-promoting or gonadotropic effect could be demonstrated upon 18 rats on a thiamin-deficient diet or upon 8 rats on a horse-meat diet. Attempts to immunize 8 young guinea pigs to thymocrescin were unsuccessful.

R.P.R.

**374. The Effect of Adrenalectomy on the Ketosis Produced in Rats by**

**Anterior Pituitary Extract.** REGINALD A. SHIPLEY, Western Reserve Univ. and Lakeside Hospital. Endocrinology, 26: 900. 1940.

Adrenalectomy did not prevent the ketonemia which was induced by an anterior pituitary extract in fasted rats; however, the response was reduced. There were fewer adrenalectomized animals than normal animals which manifested a rise in blood acetone bodies sufficient to exceed the renal threshold. Hence there were fewer in the operated series which showed ketonuria. No evidence was found for the existence of an elevated renal threshold for acetone bodies after adrenalectomy.

R.P.R.

**375. Symposium on Carbohydrate Metabolism.** C. F. CORI, SAMUEL SOSKIN, C. N. H. LONG AND F. G. YOUNG. Endocrinology, 26:

285. 1940.

An excellent review on carbohydrate metabolism. It consists of the following papers:

1. Glycogen Breakdown and Synthesis in Animal Tissues. Carl F. Cori, Dept. of Pharmacology, Washington University.
2. The Liver and Carbohydrate Metabolism. Samuel Soskin, Depts. of Metabolism and Endocrinology, Michael Reese Hospital, and Physiology, University of Chicago.
3. The Adrenal Cortex and Carbohydrate Metabolism. C. N. H. Long, B. Katzin and Edith G. Fry, Laboratory of Physiological Chemistry, Yale University.
4. The Pituitary Gland and Carbohydrate Metabolism. F. G. Young, National Institute for Medical Research.

R.P.R.

## MISCELLANEOUS

- 376. Requirements of Good Water Supplies for the Dairy Industries.** K. G. WECKEL, Dept. of Dairy Industry, Univ. of Wisconsin, Madison, Wis. Milk Dealer, 29: 7, 40-41, 66-70. April 1940.

Water supplies used in dairy plants are discussed from the following standpoints: (1) bacteriologically acceptable, (2) freedom from particles, sediment, suspensions, and oil film, (3) inert chemical properties, (4) availability in volume, (5) low temperature, (6) uniformity in composition, (7) acceptable mode of transmission.

C.J.B.

- 377. Whey Solids in Candy.** B. H. WEBB AND C. F. HUFNAGEL, Bureau of Dairy Industry, U.S.D.A., Washington, D. C. Food Research, 5: 2, 185. March-April 1940.

Sweetened condensed, Cheddar-cheese or Swiss-cheese whey was, in general, the most satisfactory of the cheese wheys for candy use. Plain condensed wheys were also suitable, but their perishable nature was a serious handicap in commercial use. Excellent candy containing up to 40 per cent of whey solids could be made, the whey replacing in part, sugar, skimmilk and corn syrup. Directions for the manufacture of various types of whey candy, including formulae are presented.

F.J.D.

- 378. Casein Plastics.** GEORGE H. BROTHER, Urbana, Ill. Ind. Eng. Chem., 32: 1, 31. 1940.

A short review of the past and present status of the casein plastics industry is presented. Casein plastic is a result of a reaction between casein and formaldehyde. Various modifications and improvements in the manufacture of casein plastics have been made since the commercial possibilities of the product were first recognized in 1897. The industry has been seriously handicapped by a lack of fundamental information on the structure and properties of proteins.

B.H.W.

- 379. Maintenance and Depreciation.** C. T. BAKER, Atlanta, Ga. Ice Cream Rev., 23: 3, 84. 1939.

To keep property and machinery in good physical condition requires vigilance and good judgment. Improper erection and insufficient or improper foundations influence the life of machinery.

J.H.E.

- 380. Production, Processing, and Storing of Fast Frozen Foods.** C. J. MEISTER, Fairmont Packing Co., Fairmont, Minn. • Ice Cream Rev., 23: 3, 34. 1939.

The handling of vegetables for fast freezing is discussed. Enzymatic action taking place during storage in the frozen state is said to be detrimental. Proper blanching of vegetables destroys the enzymes. The desir-

able temperature of storage is zero °F. and the temperature should be constant. When the temperature goes up, unless the package is hermetically sealed, the air will go out, taking some moisture with it. When the temperature is lowered, the air will pass back into the package. The package virtually "breathes" if the temperature is not kept constant. J.H.E.

- 381. Excessive Plant Operating Costs—The Penalty of Ignorance and Neglect.** C. T. BAKER, Atlanta, Ga. *Ice Cream Rev.*, 23: 4, 21. 1939.

The reason for refrigeration operating troubles in two plants are described. Inadequate condensing water and infrequent defrosting of evaporating coils were principal sources of loss of capacity. J.H.E.

- 382. Successful Farm Refrigerator Fills Many Uses.** FRED ERBACH, Chicago, Ill. *Refrig. Eng.*, 39: 6, 361. June 1940.

A combination refrigerator, one to not only cool and hold milk, freeze a slab of ice, and store frozen foods, but also preserves fresh fruits and vegetables is advocated. It is stated that there is a total market for about 1,200,000 electric units of this sort and 3,000,000 gas driven units. The author suggests essential operating features of such cabinets and presents design features. L.M.D.

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## ABSTRACTS OF LITERATURE

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New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS TO APPEAR IN THE  
JOURNAL OF DAIRY SCIENCE

- 383. Effect of Salt on the Keeping Quality of Cream.** W. J. CAULFIELD, F. E. NELSON AND W. H. MARTIN, Kansas Agricultural Experiment Station.

Four lots of 30 per cent cream to each of which salt in quantities equal to 0, 7, 10, 13 and 16 per cent of the weight of the fat-free serum was added immediately after separation, were held at 60, 70, 82 and 90° F. for 10-day periods. Changes in acidity, formol titration and grade were followed. Changes in the bacterial flora of two of the four lots were followed by direct microscopic observations.

Deterioration of the creams was definitely retarded by the addition of salt. The amount of salt necessary to effectively prevent appreciable deterioration was found to be dependent upon time and temperature of storage. The results indicate that not less than 10 per cent salt (serum basis) is required to prevent cream from becoming second grade when held for 10 days at 60 or 70° F. and for 5 days at 82 and 90° F.

The addition of 13 per cent salt (serum basis) to cream held at 70° F. for 3 or more days before salt was added did not prevent further deterioration of the cream. Thus the method is largely limited to farm use.

Butter churned from cream to which 13 per cent salt (serum basis) was added at the beginning of a 10-day storage period at 70° F. scored two to five points higher than did butter produced from control lots of the same cream held under similar conditions without salt.

When a modified Babcock test procedure was used, results which compared favorably with the calculated butterfat percentages were obtained.

The data indicate that the improvement of the keeping quality of cream by the addition of salt has definite merit.

- 384. The Effect of Alfalfa Lipids upon the Progress of Sweet Clover Poisoning in Cattle.** W. A. KING, H. A. CAMPBELL, I. W. RUPEL, P. H. PHILLIPS AND G. BOHSTEDT, Departments of Biochemistry and Dairy Husbandry, University of Wisconsin, Madison.

A study of the effect of alfalfa lipids upon the progress of sweet clover poisoning in cattle has been made and the following results obtained.

Ten per cent of the ration of growing cattle was made up of toxic sweet clover and fed without harm for a period of 3½ months.

Animals with a prolonged clotting time developed an increased number of blood platelets. There was no change in the fibrin, hemoglobin, or serum calcium in these cases.

Crude petroleum ether extracts of alfalfa hay fed at a level equivalent to 60 per cent of the toxic sweet clover in the ration brought about a favorable remedial response in sweet clover poisoned young cattle. Evidence adduced from the separate effects upon whole blood clotting time and prothrombin clotting time, the administration of bile salts alone and with alfalfa lipids, and the difference in rate of return to normal between the prothrombin and blood clotting times when the toxic hay was withdrawn from the ration indicates that one or more factors other than prothrombin were involved in the restoration of the normal blood clotting mechanism of the sweet clover poisoned bovine.

**385. The Effect of Added Egg Phospholipids on the Nutritive Value of Certain Vegetable Oils.** E. J. SCHANTZ, R. K. BOUTWELL, C. A. ELVEHJEM AND E. B. HART, Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

It was recently reported from this laboratory<sup>1</sup> that butter fat has a higher nutritive value for growth in weanling rats than certain vegetable oils when homogenized into mineralized skimmed milk and supplemented with all the known essential fat soluble vitamins. The difference in the nutritive value was not found to be due to factors contained in the non-saponifiable fraction of butter fat.<sup>1</sup> However, since the phospholipids are decomposed upon saponification it appeared possible that the difference in the nutritive value of butter fat and the vegetable oils fed might be due to some particular phospholipid contained in the butter fat which was not contained in the vegetable oils.

Addition of 0.25 per cent and 0.5 per cent of egg lecithin to corn oil or coconut oil improved the nutritive value of these oils slightly but not enough to make them equal to butter fat when they were homogenized into mineralized skimmed milk at a level of 4 per cent and fed to weanling rats.

Sphingomyelin sphingosine sulfate, and ethanolamine had no effect on the nutritive value of corn oil, but choline, in the case of the females, seemed to improve it slightly.

**386. The Nutritive Value of the Fatty Acid Fractions of Butter Fat.** E. J. SCHANTZ, R. K. BOUTWELL, C. A. ELVEHJEM AND E. B. HART, Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

In a previous paper<sup>1</sup> it was reported that butter fat homogenized into mineralized skim milk gave better growth of weanling rats than certain

<sup>1</sup> Schantz, E. J., Elvehjem, C. A., and Hart, E. B. The comparative nutritive value of butter fat and certain vegetable oils. *J. DAIRY SCIENCE* 23, 181, 1940.

vegetable oils homogenized into skim milk and fed under the same conditions with ample carotene, irradiation,  $\alpha$ -tocopherol and minerals added in all cases. The superiority of butter fat for growth was not found to be due to factors contained in the non-saponifiable fraction of butter or to be due to compounds such as lecithin, choline, sphingomyelin, or sphingosine.

The fatty acids responsible for the superior growth of young rats obtained on butter fat as compared with certain vegetable oils homogenized into skim milk with all of the known essential fat soluble vitamins added, apparently lie in the saturated fraction of butter fat.

When the fatty acids of butter fat were separated into the volatile acids by steam distillation and into the unsaturated and saturated acids as lead soaps and the triglycerides of these fractions fed in corn oil in approximately the composition found in butter the *saturated* fraction with corn oil was found to be a little superior to butter fat while the other two fractions compared favorably with corn oil.

**387. The Length of the Intestine of Calves and Its Bearing on the Absorption of the Nutrients from the Chyme.** DWIGHT ESPE AND C. Y. CANNON, Iowa State College, Ames, Iowa.

Data are presented indicating that the small intestine of the living calf is about seven times the body length, or about one-third the post-mortem length. The large intestine does not show as great a difference in length between the living and post-mortem stages as does the small intestine.

Variations in the ratio between body length and length of intestine depend more on individuality than upon the age of the calf.

**388. The Specificity of the Lactogenic Hormone in the Initiation of Lactation.** A. J. BERGMAN AND C. W. TURNER, Dept. Dairy Husbandry, Univ. of Missouri, Columbia, Missouri.

A study is reported with the "lactogenic hormone" and the "thyrotropic and other hormone" fractions of the anterior pituitary on lactogenesis in the pseudo-pregnant rabbit. The injection of small amounts of the "lactogenic hormone" fraction was necessary to produce abundant lactation, (++) to (+++) glands. Extracts rich in the "thyrotropic and other hormones," but containing only traces of the lactogenic hormone did not possess the ability to initiate lactation in doses as high as could be tolerated. These results are taken to indicate that the primary function of the lactogenic hormone, which also possesses the ability to proliferate the pigeon crop gland, is to initiate and maintain established lactation, while the "thyrotropic and other hormones" fraction has only a supplementary effect on established lactation.

**389. Distribution of *Pseudomonas fragi*. H. B. MORRISON AND B. W. HAMMER, Iowa State College, Ames, Iowa.**

*Pseudomonas fragi* was found in 16.5 per cent of 176 lots of milk delivered by 14 producers to an Iowa milk plant. It was not detected in 17 lots delivered in June to a Kentucky milk plant but was found in 40.0 per cent of 40 deliveries in December to the same plant. Samples of defective dairy products, especially those criticized as rancid or as having a May apple odor, commonly yielded the organism.

In general, dairy plant equipment was relatively free of the organism. An appreciable percentage (9.7 per cent) of the Iowa dairy plant water supplies yielded the organism. In Iowa a large proportion (51.8 per cent) of samples of dirt and other materials or equipment likely to come in contact with or be contaminated by dirt was found to harbor *Ps. fragi*. In Kentucky relatively few (4.1 per cent) similar samples obtained in summer yielded it, but it was found in a considerable proportion (37.4 per cent) of the samples obtained in December.

*Ps. fragi* was found in 25 (71.4 per cent) of 35 samples of barnyard soil obtained from various states. It was present in a larger percentage of samples from the eastern states (90.9 per cent) than from the western states (38.5 per cent).

The wide distribution of *Ps. fragi* on farms emphasizes the importance of farms as a source of the organism.

**390. A Study of Fresh and Frozen Plain, Superheated and Sweetened Condensed Skimmilk for Ice Cream. L. K. CROWE AND HARRY H. WINN, Dairy Husbandry Department, University of Nebraska, Lincoln, Nebr.**

Plain, superheated and sweetened condensed skimmilk made from the same lot of skimmilk was used fresh and after one, two, and three months storage at 0° F. as the source of added serum solids in ice cream. The three types of condensed skimmilk were satisfactory sources of serum solids when fresh and after storage. No benefits were derived from superheating condensed skimmilk that was stored frozen. The protein in superheated condensed showed precipitation after one month at 0° F. Storage of any of the three types of condensed skimmilk had no consistent appreciable effect upon the protein stability of the ice cream mix in which they were used.

Viscosity of the ice cream mixes was not consistently affected by the freezing and storing of the condensed skimmilk. The viscosity of the ice cream mixes made with superheated condensed skimmilk was but slightly higher than that of ice cream mixes made with the other two types of condensed skimmilk.

Average whipping curves indicate that ice cream mixes made with fresh plain condensed skimmilk whipped to 100 per cent overrun faster than ice

cream mixes made with fresh condensed skimmilk of the other two types. The time required to reach 100 per cent overrun for ice cream mixes made with the three types of condensed skimmilk stored frozen two and three months was less than the time required for mixes made with fresh condensed skimmilk.

There was no appreciable difference in the flavor, body or texture scores which could be attributed to the type of condensed skimmilk used or whether it was the fresh or frozen product.

Ice cream made with fresh superheated condensed skimmilk was slower in melting than ice cream made with either of the other two types of condensed. Ice cream made with superheated condensed skimmilk gave the least foam on melting. Freezing and storing plain and superheated condensed skimmilk increased the amount of foam in the melted ice cream whereas the opposite was true for sweetened condensed skimmilk. Ice cream made with stored frozen superheated condensed skimmilk was frequently criticized for a slight curdy appearance on melting.

**391. A Semimicro-Kjeldahl Method for the Determination of Total Nitrogen in Milk.** S. G. MENESEE AND O. R. OVERMAN, Univ. of Illinois, Urbana, Ill.

Recent research work in this laboratory pertaining to the nitrogen distribution in dairy products made it desirable to develop a practical method for determining small amounts of nitrogen.

The purpose of this experiment was: (1) to design a semimicro-Kjeldahl apparatus, (2) to compare the efficiency of several digestion catalysts and (3) to compare both 0.02 N sulphuric acid and boric acid solutions as the ammonia receiving agents.

Copper sulphate and selenium oxychloride, mercuric oxide and selenium oxychloride, and mercuric oxide alone were used as the digestion catalysts. These three catalysts gave comparable results when used to analyze milk for total nitrogen. Mercuric oxide is preferred as a catalyst because the literature for the most part, indicates that selenium and its combinations cause low results for nitrogen.

A 2 per cent boric acid solution is preferred as the ammonia receiving agent with a methyl red-methylene blue combination as the indicator. The boric acid solution eliminates the use of 0.02 N sodium hydroxide solution (for back titrating the standard acid) which is very sensitive to carbon dioxide and requires frequent restandardization.

The semimicro-Kjeldahl method is well adapted to routine analysis, it saves time, and reduces the cost of reagents per determination.

The results obtained with the semimicro-Kjeldahl check very closely with those obtained by the Official Method.

## BOOK REVIEWS

392. **Food Industries Manual; 10th Edition.** Published by Leonard Hill Limited, London. Distributed in U. S. by Chemical Publishing Co., Inc., New York. 400 pages, price \$4.00.

This is a technical and commercial compendium of the manufacture, preserving, packing and storage of various food products. The volume aims at collecting information which has become established and standardized in the various food industries. It succeeds best in doing this in the case of milling, baking, sugar confectionery, chocolate, and canning. The section devoted to the dairy industry is limited and chiefly contains formulae for various common dairy calculations, some test procedures and a few tables on the composition of dairy products. The volume contains an extensive list of books likely to interest those engaged in the food process industries, but it has not been brought up to date. The subject matter is so broad that it has been necessary to condense greatly most of the subjects dealt with.

J.H.E.

393. **Dairy profit.** WILBUR J. FRASER, Interstate Printers and Publishers, Danville, Ill. 270 pages, Illustrated. Price \$1.80. 1940.

"Dairy Profit" is a valuable contribution to dairy literature.

Although much of the material has appeared previously in periodicals, it is here grouped and classified and Professor Fraser has added much of his own philosophy about agriculture and especially dairying and dairy people.

The author emphasizes the need for planning farm and dairy operations on a long time basis.

The subject matter of the book deals with the problems of feeding and management and crop production necessary to successful dairy farm operation.

Professor Fraser discusses the principles of feeding and management in simple straight forward terms and in most instances illustrates the effect of good management by a story of some good farmer. Throughout the book he stresses the need of proportionality and shows how disastrous it is to do part of the job well in all particulars save one and then miss the goal of success.

The word "profit" in the title is used in its broadest sense for the author in the last chapters of the book shows the need of planned recreation. He discourages the unnecessary drudgery which may be needed to merely accumulate large holdings of land and money. This idea is indicated by the title of one of the chapters, "What Shall it Profit a Man."

This book should be of special interest to farmers and teachers; to farmers, it gives suggestions and encouragement; to the teachers it will give a wealth of illustrations to vitalize subject matter.

C.L.Blackman

## BACTERIOLOGY

- 394. The Action of Chemical Disinfectants on Bacteriophages for the Lactic Streptococci.** G. J. E. HUNTER AND H. R. WHITEHEAD. The Dairy Research Institute, Palmerston North, New Zealand. *J. Dairy Res.*, 11: 62-66. 1940.

The times needed for the complete inactivation of bacteriophages for the lactic streptococci by hypochlorite, potassium permanganate, hydrogen peroxide, formaldehyde, mercuric chloride, alcohol, and phenol in various strengths determined. The phage preparations consisted essentially of whey. Inactivation was determined by failure of the phage chemical mixture to prevent the coagulation of milk inoculated with the susceptible organism.

When all the phage preparations were adjusted to a common protein percentage of 0.49 per cent, it was found that all of the eight phage races studied were destroyed by exposure to 0.05 per cent active chlorine within 1 minute. Active chlorine and permanganate were by far the most effective of the disinfectants.

Hydrogen and hydroxyl ions were found to inactivate phage when they were present in sufficient concentrations, but their effects between pH values of 4 and 7 were negligible.

S.T.C.

- 395. The Influence of Various Factors on the Fermentation End-Products of the Heterofermentative Streptococci.** C. C. THIEL, The Nat'l Inst. for Res. in Dairying, Univ. of Reading, Reading, England. *J. Dairy Res.*, 11: 51-61. 1940.

The influence of temperature, pH, oxygen tension and yeast autolysate on the production of by-products and the ratios of the by-products formed to the sugar utilized and lactic acid produced in milk by the heterofermentative lactic acid streptococci were investigated.

The total production of lactic acid was increased by anaerobic conditions and low temperature. The ratio of lactic acid formed to sugar utilized was increased by anaerobic conditions and the presence of chalk.

The total amount of acetic acid was higher in the presence of chalk at the lower temperatures and in yeast milk, but was decreased by anaerobic conditions. The ratio of acetic acid both to sugar utilized and to lactic acid formed was smaller at lower temperatures, in the presence of "growth factors" and chalk and under anaerobic conditions.

The total alcohol production was high when yeast or chalk was added to milk, under anaerobic conditions and at lower temperatures, and similarly the ratio of alcohol formed to sugar utilized and lactic acid produced was increased.

Hydrolysis of the residual lactose occurred in all cultures of *Str. citrovorus* and with some of the other streptococci in cultures to which chalk had been added.

S.T.C.

- 396. Studies on the Methylene-Blue Reduction Test. II. Comparison between the Old and the Modified Methods.** T. MATUSZEWSKI AND J. SUPINSKA, Institute of Fermentative Industry and Agricultural Bacteriology, Warsaw, Poland. *J. Dairy Res.*, 11: 43-50. 1940.

The old and modified (inversion of tubes at 30 minute intervals) methods of the methylene blue reduction test were compared using 185 samples of raw milk. The deviations in the reduction time, corresponding to a given initial number of cells, are smaller by the modified than by the old method, and the average reduction time is shorter. It is suggested that this is due to a more uniform distribution of the bacteria in the milk following the inversion of the tube.

The average coefficient of multiplication in the old method was 0.660 and in the modified method 0.885, showing that the shortening of the reduction time in the modified method is due to stimulation of bacterial growth.

S.T.C.

- 397. Haemolytic Organism Isolated from Pasteurized Cream.** L. O'DROMA, Dept. of Dairy Bacteriology, University College, Cork, Ireland. *J. Dairy Res.*, 11: 37-42. 1940.

A description is given of a weakly haemolytic organism, which first appeared as a contaminant, in the form of pin point colonies, in agar plates inoculated with dilutions of a cream which had been subjected to partial processing in a butter churn. The majority of its characteristics showed it to be a resistant strain of *Str. thermophilis*, although it resembled in many respects certain strains of *Str. Bovis*.

S.T.C.

## BREEDING

- 398. Rindviehzucht in Schweden.** IVAR JOHANSSON. *Züchtungskunde* 15 (4): 97-104. 6 figs. 1940.

Three breeds of cattle are bred pure in Sweden. The Black and White which is similar to the Holstein-Friesian is most abundant in southern Sweden. The Red and White which is somewhat like the Shorthorn and Ayrshire is the most popular in middle Sweden. A smaller hornless native race is more abundant in northern Sweden. Pictures of the Red and White and of white hornless animals are included. Cow testing associations began in 1898. About 18 per cent (350,000 head) of the milking cows are now on test in these associations. About 50,000 other cows are on test in creamery

associations where the owner weighs the milk and takes the samples himself but the testing is done at the nearest creamery. These creamery tests are not recognized in the conduct of the herdbooks or when awarding prizes at the fairs but are thought worth making for the owner to use in controlling his feeding operations and in culling. Progeny tests of bulls generally require at least ten comparisons between daughters and dams but in certain circumstances six such comparisons justify evaluating a bull. Some government aid is given to the cow testing associations and also to the 2,400 bull associations. Members of the latter own 286,000 cows. There are no specialized beef breeds in Sweden and no cattle are now used for draft purposes. Breeding is aimed at a milk-flesh ideal with the milk being given more emphasis, especially in the northern districts. The total number of cows has not changed much in recent years but production has increased. Improved breeding and better management have both been responsible for this. About eight per cent of the feed for dairy cows is imported. Various governmental subsidies or control measures have kept the domestic price for butter higher than the price for butter exported. Some of these last circumstances may have changed since the outbreak of the present war.

. J.L.L.

**399. Die Auswertung der Herdbücher in den Fleckviehzuchtgebieten unter besonderer Berücksichtigung der Leistungsvererbung durch den Bullen. HANS BIEGERT. Züchtungskunde 15 (4) : 105-119. 1940.**

The author gives first a general discussion about the purposes and uses of herdbooks, the nature of the data they do or should include, methods of evaluating inheritance for milk production, etc. The data studied are from the herdbooks of the Ried region in central Baden and involve evaluating the production or inheritance of about 140 bulls and 4,000 cows. All of the cattle belong to the Fleckvieh race (similar to the Simmenthaler). The agriculture of this region is characterized by small holdings with usually not more than three or four cows per farm and consequently the extensive use of community bulls and such cooperative measures. Under such conditions the author believes it unwise to refer each record to the herd average, as has been recommended for regions where the herds are very large and the data extend over many years. Also he concludes that the year-to-year variation need not be considered in types of agriculture in which little use is made of pasture. Daughter and dam were tested in the same herd in about 95 per cent of the cases. Age corrections are avoided by not including the first two lactations in the daughter-dam comparisons. He uses averages of the third and fourth lactations and later ones, if any. Each sire is evaluated according to the customary "heredity grid" diagram which is like an intra-sire daughter-dam correlation. The diagram also shows the numbers of the

daughters which exceed or fall below their dams and the number which exceed or fall below the average for the community in which that bull was kept. Daughters without tested dams or without third and fourth lactations are indicated in the diagram but are not included in the proof. Ten daughter-dam comparisons are considered the minimum for proving a sire. Corrections for the amount of draft work performed by the dam or by the daughter are discussed but are considered unsatisfactory. An actual pedigree is given as a model of what would be expected in pedigrees giving only the most useful information. No new genetic theory is involved. This is a discussion with actual examples of what can be done toward the proving of dairy sires in communities where the herds are small and the cooperative use of dairy sires is extensive. It also includes a number of brief statements of opinion as to which external circumstances are practically worth correction and concludes that in this region age is almost the only one of these.

J.L.L.

## BUTTER

- 400. Crumbly, Sticky Butter.** C. H. PARSONS, Swift & Co., Chicago. Nat'l Butter and Cheese J., 31: 4, 18. 1940.

Crumbly butter is difficult to cut into patties and breaks easily when cold. Composition of butter fat and manufacturing procedures influence the extent of the defect. Butter manufacturers cannot control feeds but may relieve the condition by careful manufacturing methods. Control of temperature of churning and temperature of wash water as suggested by Coulter and Combs were tried on the commercial scale in 9 states. Butter was tested by penetrometer, and by cutting and spreading tests. The treatments improved but did not wholly overcome severe crumbly conditions. The auger type printing machine improved body. There is a possibility of further improvement by proper adjustment of time and temperature of holding butter before printing.

W.V.P.

- 401. Weedy Flavored Cream—Its Relation to the Butter Industry.** P. A. DOWNS, Univ. of Nebraska, Lincoln, Nebr. Nat'l Butter and Cheese J., 31: 5, 12. May 1940.

Flavors of milk and cream may come from feeds and weeds eaten by cows or from odors breathed by the cow and absorbed by the blood stream. *Thlaspi arvense*, called Pennycress, Frenchweed or stinkweed, belongs to the mustard family and now is found from Canada south to central California and east to eastern Minnesota. As little as 90 to 150 gms. of seed or 500 gms. of green forage eaten by the cow will taint the milk. Wild onion or garlic plants are common causes of tainted milk. "Pepper grass" is believed to cause a characteristic flavor but more definite information is

needed to be sure of its effect. This information is being obtained by the combined efforts of the Research Committee of the American Butter Institute of Chicago, and a Sub-committee of the Quality Committee of the American Dairy Science Association.

W.V.P.

## CHEESE

### 402. The Measurement and Significance of pH Values in Cheesemaking.

J. G. DAVIS AND C. C. THIEL, The Nat'l Inst. for Res. in Dairying, Univ. of Reading, Reading, England. *J. Dairy Res.*, 11: 71-78. 1940.

A colorimetric dilution method for the determination of pH values in cheesemaking is recommended, using bromthymol blue as the indicator for milk and whey of pH greater than 5.8 and B. D. H. 5560 indicator for whey of pH below 5.8. The authors consider that titratable acidities and pH values each afford an incomplete picture of the working of a curd. Together they afford a valuable indication of the normality or otherwise of the making process.

S.T.C.

### 403. Cream Cheese as a Base for Spreads. C. R. BAKER. Nat'l Butter and Cheese J., 31: 6, 26. June 1940.

A method of making "Cream cheese" with six per cent fat in the mix is outlined. The curd may be mixed with ripened cheese, pickles, olives or pimentos. Emulsifying salts and a stabilizing agent are added to the cheese mixture which is cooked to about 170° F. before placing in glass jars.

W.V.P.

## CHEMISTRY

### 404. Biennial Reviews of the Progress of Dairy Science. Section C. Dairy Chemistry. *J. Dairy Res.*, 11: 84-111. 1940.

An excellent review of the progress of dairy chemistry from the middle of 1937 to the middle of 1939.

S.T.C.

### 405. The Determination of Vitamin D in Food Substances Containing Phosphorus. KATHARINE COWARD AND ELSIE KASSNER, College of Pharmaceutical Society, London. *Biochem. J.*, 34: 538. 1940.

In this paper a reinvestigation of the effect of giving vitamin D plus phosphate to rats fed on a rachitogenic diet of high-Ca, low-P content was reported. In six separate experiments the average healings produced by (a) 10 units of vitamin D, (b) a dose of potassium phosphate, and (c) 10 units of yitamin D plus a dose of phosphate were compared. The phosphate dose of groups (b) and (c) ranged from 60 to 1380 mg. per rat. In another series

similarly arranged the vitamin D dose was 5 units, and the phosphate dose ranged 460, 920 and 1380 mg. per rat. The lower doses of phosphate alone were practically without effect on calcification but all doses affected the calcification brought about by either 5 or 10 units of vitamin D. The curves of response to (a) 10 units of vitamin D plus graded doses of phosphate, (b) 5 units of vitamin D plus graded doses of phosphate, and (c) doses of phosphate alone were all logarithmic and of similar slope, (a) being highest, and (c) lowest of the various heights. Giving both vitamin D and phosphate to rats receiving a rachitogenic diet of high-Ca, low-P content has a multiplicative effect on healing, not an additive one. It is suggested the only practical method of testing a food product containing sufficient phosphorous in the dose to alter the Ca-P ratio of the diet is to extract the ether soluble portion after saponification and determine the vitamin D in the extract.

K.G.W.

**406. Studies on the Chemistry of the Fatty Acids. VI. The Application of Crystallization Methods to the Isolation of Arachidonic Acid, with a Comparison of the Properties of this Acid Prepared by Crystallization and by Debromination. Observations on the Structure of Arachidonic Acid.** G. Y. SHENOWARA AND J. B. BROWN, Lab. of Physiological Chem., Ohio State Univ., Columbus, O. J. Biol. Chem., 134: 331. 1940.

Crystallization procedures at low temperatures were applied to the methyl esters from the fatty acids of suprarenal phosphatides. The methyl arachidonate obtained by this method was compared, for the constants, with the ester prepared by the debromination procedure. The differences in constants observed are believed due to the presence of isomers in the product obtained by the chemical means. A tentative formula for the arachidonic acid is suggested, based on physico-chemico measurements. The arachidonic acid occurring in adrenal phosphatides is suggested as  $\Delta$ -6-10-14-18  $\alpha$  icosatetrenoic acid.

$\text{CH}_3 - \text{CH} = \text{CH} - (\text{CH}_2)_2 - \text{CH} = \text{CH} - (\text{CH}_2)_2 - \text{CH} = \text{CH} - (\text{CH}_2)_4 - \text{COOH}$ . This information is of interest because of increasing attention to the value of fatty acids in nutrition.

K.G.W.

**407. The Estimation of Riboflavin. 1. A new biological method. 2. The estimation of riboflavin in milk; comparison of fluorimetric and biological tests. 3. Statistical analysis of the data.** M. M. EL SADR, T. F. MACRAE AND C. ELIZABETH WORK, Div. of Nutrition, Lester Institute, London; K. M. HENRY, J. HOUSTON AND S. K. KON, Nat'l. Institute for Research in Dairying, Univ. of Reading; AND J. O. IRWIN, Medical Research Council's Statistical Staff. Biochem. J., 34: 601. 1940.

The first phase of the paper is a concise review of the techniques available for measuring the riboflavin content of foods, and the results of these methods. The second phase is a report of a study on the biological assay method wherein a basal ration plus various supplements were fed the animals. A method was suggested for use whereby young rats are fed a diet complete in other respects plus a liver charcoal filtrate as a source of the B<sub>2</sub> vitamins. Inasmuch as the rats showed growth responses when fed graded doses of riboflavin, it is believed an excellent method for assay purposes.

In the third phase a comparison was made between the fluorimetric method of measurement of riboflavin and the bioassay method. When the milk is fed at lower levels yielding animal-weight gains of 10-15 grams a week, biological findings are in good agreement with fluorimetric tests. In the fourth phase, the bioassay results of two laboratories were put to statistical review. The results of the data show that the slope of the standard curve of data by the one laboratory is steeper than the slope for standard of the second laboratory. Because different slopes for the standard curve occur in different laboratories, it is suggested that at least two doses of standard and two doses of unknown preparation be employed in routine testing.

K.G.W.

- 408. The Composition of Dolphin Milk.** LILLIAN EICHELBERGER, E. S. FLETCHER, JR., E. M. K. GEILING AND B. J. VOS, JR., Lasker Foundation for Medical Research and the Depts. of Medicine, Pharmacology, and Physiology, Univ. of Chicago. *J. Biol. Chem.*, 134: 171. 1940.

The data presented in this study were obtained in a manner unlike that employed for other studies; the samples were obtained from *live* animals, three bottle-nose dolphins and in addition, one spotted dolphin 1.5 hours after it had been harpooned. Dolphin milk was found to be high in fat (108-180 gm. per liter) and protein (94.2-111 gm. per liter) and low in lactose (3.9-7.7 gm. per liter).

K.G.W.

- 409. The Determination of pH in Milk and Whey by Means of Colour Indicator Paper.** L. SEEKLES, The Laboratory for Veterinary Chemistry, State University, Utrecht, Holland. *J. Dairy Res.*, 11: 79-83. 1940.

The author suggests the use of lypan paper M25 for pH measurements in milk and whey in the pH range 5.4-6.6. As a rule the error was found not to exceed 0.1 pH. In very acid milk and whey ( $\text{pH} < 5.4$ ) lypan paper of the L series is recommended. Generally the deviation was found not to exceed 0.2 pH. Lypan paper can not be used in alkaline milk and whey.

S.T.C.

**410. A Rapid Method for the Separation of Serum Albumin and Globulin.**

GEORGE B. KINGSLEY, Div. of Biochem., Labs., Phila. Genrl. Hospital, Phila., Pa. J. Biol. Chem., 133: 731. 1940.

A rapid, accurate method for the separation of serum albumin and globulin is based on the use of ether to decrease the density of globulin precipitated by sodium sulphate. After ether extraction and brief centrifugation, globulin separates in a compact layer at the bottom of the ether phase above the sodium sulfate solution. No preliminary period of standing is required.

K.G.W.

**411. Composition of Lactic Acid and the Production of a Highly Concentrated Acid.** PAUL D. WATSON, Res. Labs., Bur. Dairy Ind., U. S. Dept. of Agr. Ind. Eng. Chem., 32: 399. 1940.

Lactic acid with a total acidity of about 100 per cent consists of varying proportions of lactic acid (free), lactylactic acid (anhydride), lactide and water.

The composition of lactic acid of different strengths varies widely, and the composition of a single acid changes slowly until equilibrium is reached. Lactic acid (U.S.P. 85 per cent) contains only a trace of lactide; the 100 per cent acid contains about one per cent lactide. The amount of lactylactic acid present may vary from about zero to about 90 per cent, dilute lactic acid containing little or none. Smooth curves are obtained when the analytical results obtained on stabilized acids are plotted.

At present concentrated lactic acid is not available in concentrations above 86 per cent. This study has resulted in a process for the production of concentrated lactic acid with a total acidity of about 105 per cent expressed as lactic acid. This concentrated acid is an anhydrous, viscous, water white liquid, and it is thought that it may have considerable industrial value.

Author's Abstract

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

**412. Density of Dry Milk Solids (Skimmilk).** O. E. STAMBERG AND C. H. BAILEY, Univ. of Minnesota, St. Paul, Minn. Food Research, 5: 3, p. 275. May-June 1940.

The authors define a term "density index" as ten times the amount of sedimentation obtained, by shaking 7 grams of dry skimmilk with a naphtha and carbon tetrachloride mixture (density 1.250 at 25° C. or 77° F.) in a graduated 50 cc. conical centrifuge tube, after 45 minutes settling. Their observations indicate that there is a great variation in the density indices of spray dried skimmilk due to the variation in amount of occluded air. This in turn was found to vary with the process of manufacture and type of equipment. No air was found in roller dried skimmilk and less air was

found in spray dried skimmilk when a higher preheating treatment was used. The authors indicate that the density index might be used by manufacturers as a test for uniformity of product. While no mention is made directly concerning it, the index appears to also offer a means of distinguishing between spray dried and roller dried skimmilk.

F.J.D.

## FEEDS AND FEEDING

**413. Milk and Butterfat Production by Dairy Cows on Four Different Planes of Feeding.** R. R. GRAVES, GEORGE BOTEMAN AND J. B. SHEPPARD, all of the Bureau of Dairy Industry, AND GEORGE B. CAINE, Utah State Agr. College. U.S.D.A. Tech. Bul. 724, 36 pages. April 1940.

Twelve Holstein cows were fed the following 4 different rations in 4 lactation periods and the production records calculated to maturity.

1. Full grain ration—consisting of alfalfa hay, corn silage, pasture in season and one pound of the grain mixture per pound of fat produced per week. Grain mixture was 2 parts barley, 1 part oats and 1 part bran.
2. Alfalfa hay alone or pasture alone in season.
3. Same as (2) except ground barley was fed at the rate of one pound to an average of 6.03 pounds of milk.
4. Alfalfa hay and pasture with the addition of corn silage.

Ration 1 was fed prior to the other rations which did not follow in order. On the basis of ration No. 1 being 100 per cent, ration No. 2 produced 69.75 and 65.77 per cent as much milk and butterfat respectively; ration No. 3 produced 86.03 and 80.24 per cent as much milk and butterfat respectively.

The average dry matter consumed daily per cow during the winter was 30.6 pounds on alfalfa alone, 32.85 pounds on alfalfa and restricted grain and 32.76 pounds on alfalfa and corn silage.

W.E.P.

**414. Roughage Feeding of Dairy Cattle.** H. S. WILLARD, Univ. of Wyoming, Laramie, Wyoming. Bul. 237, May 1940.

Barley as the grain supplement to alfalfa hay and pasture was compared to alfalfa and pasture only for milking cows. The effect of several consecutive years of no grain feeding was also studied. From 2 to 12 pounds of barley per head per day was found to have very little effect upon lowering the hay consumption.

When Holstein cows had a daily capacity of 30 to 40 pounds of milk at the peak there was little increase in production when barley was fed with good hay and pasture. Cows with greater capacities at peak production benefit proportionally from grain feeding. When no grain is fed the greater the peak production capacity is, the more rapid the decline in daily milk production. Cows with 50 to 60 pounds peak production capacities produced

327 pounds fat in 305 days mature equivalent without grain and 410 pounds with barley supplement. Cows with 40 to 50 pounds daily peak capacities produced 317 and 320 pounds fat on the mature equivalent in 305 days for no grain and grain respectively. When the peak production capacity was 30 to 40 pounds the respective 305 day mature equivalent fat yield for no grain and grain was 240 and 214 pounds.

W.E.P.

**415. Rate of Growth by Dairy Calves and Heifers on Different Rations.**

R. R. GRAVES AND J. R. DAWSON, Bureau of Dairy Industry, Washington, D. C.; D. V. KOPLAND, Huntley, Mont.; A. G. VAN HORN, Lewisburg, Tenn.; and S. L. CATHCORT, Columbia, S. C. U.S.D.A. Cir. No. 560, 24 pages. 1940.

Holstein heifers were fed skim milk until 6, 12, 18 and 24 months of age. No difference was found in the rate of growth. The longer periods of skim-milk feeding resulted in a somewhat higher breeding efficiency. A slightly higher milk production was observed for the heifers fed skim milk for the longer periods which was not attributable to inheritance. At Woodward, Oklahoma, with 3 groups of 3 Holstein heifers each, it was found that satisfactory growth was secured on winter rations consisting of sumac sorgo silage and 1 pound cottonseed meal; sumac sorgo silage and 2 pounds cottonseed meal and 6 pounds alfalfa hay; sumac sorgo silage and 2 pounds grain mixture.

Jersey heifers were found to make satisfactory growth from 12 months of age when fed unlimited amounts of machine dried legume hay during the winter and good pasture during the summer. Thirteen Jersey heifers fed machine dried hay exclusively and without pasture from 12 months to 18 months of age made satisfactory gains. The daily hay consumption per head ranged from an average of 10.6 pounds at 13 months to 15 pounds at 18 months of age.

W.E.P.

**FOOD VALUE OF DAIRY PRODUCTS****416. A Physiological Explanation of the Therapeutic Value of Milk.**

CHARLES F. NELSON, M.D., Nelson Clinic, Beverly Hills, Calif. The Assn. Bull. Intern. Assn. of Milk Dealers. 32nd year. No. 16: 393-398. May 1940.

The results of physio-chemical tests show that 68 per cent of all people are below 10 mgs. of blood calcium, whereas the author considers 10.5-12 mgs. per cent (equivalent to 12 mgs. per 100 ml. of blood) as the optimum. He further states that nearly all cows are deficient in calcium. The serum calcium and phosphorus determinations of 100 women on date of delivery showed 85 per cent were deficient in phosphorus. In order to maintain a normal calcium concentration in the blood, it is necessary to maintain a nor-

mal blood phosphorus concentration. Low blood calcium also results from too high phosphorus, in which event phosphorus intake must be reduced until the calcium-phosphorus serum concentration ratio is 3 to 1. If it is possible to increase the amount of calcium in cows' milk in addition to calcium containing foods, especially alfalfa, oats, etc., it is necessary to add some form of calcium medication to the food mixtures either in the form of decomposed limestone, calcium carbonate or calcium gluconate. A blood calcium content of 12 mgs. per cent is desired.

E.F.G.

- 417. The Vitamin A and Carotene Content of Shorthorn Colostrum.** K. M. HENRY, J. HOUSTON AND S. K. KON, Nat'l Inst. for Res. in Dairying, Univ. of Reading, Reading, England. *J. Dairy Res.*, 11: 1-8. 1940.

The concentration of carotene and vitamin A in colostrum and colostral fat and total yield of these substances in successive milkings was studied for four Shorthorn cows and nine heifers. The concentration of vitamin A in the first colostrum ranged from 8160 to 820 Moore blue units per 100 g. and that of carotene from 2026 to 411 Moore yellow units per 100 g. The highest and lowest concentrations and yields of vitamin A and carotene respectively in samples of colostrum and later milk were in the ratios: per g. of colostrum (milk) 35:1 and 65:1; per g. of fat 27:1 and 34:1; calculated on daily yield 31:1 and 65:1.

Access to pasture before calving appeared to have no effect on the secretion of vitamin A in colostrum but increased the output of carotene.

S.T.C.

- 418. The Effect of Commercial Pasteurization and Sterilization on the Vitamin B<sub>1</sub> and Riboflavin Content of Milk as Measured by Chemical Methods.** J. HOUSTON, S. K. KON AND S. Y. THOMPSON, The Nat'l Inst. for Res. in Dairying, Univ. of Reading, Reading, England. *J. Dairy Res.*, 11: 67-70. 1940.

Fluorimetric tests applied to commercially pasteurized and commercially sterilized milk showed that in the former some 10 per cent and in the latter up to 50 per cent of vitamin B<sub>1</sub> was destroyed in the course of the heat treatment.

Riboflavin withstood both treatments without loss.

S.T.C.

- 419. The Problem of Variations in the Growth-Promoting Value of Milk for Rats.** S. BARTLETT, K. M. HENRY AND S. K. KON, The Nat'l Inst. for Res. in Dairying, Univ. of Reading, Reading, England. *J. Dairy Res.*, 11: 22-36. 1940.

The growth-promoting properties of milk from three Shorthorn cows grazed on the best available permanent pasture was compared to that pro-

duced by three other cows of similar lactational history which had been on winter rations for 6 months at the start of the experiment and continued to be stall-fed. By suitable blending of morning and evening milkings the fat content of the milk from both groups of cows was adjusted daily to a common level. A part of each milk was then holder-pasteurized.

Four groups of specially prepared litter-mate male rats were fed *ad lib.*, for a period of eight weeks, on raw and pasteurized milks, both being supplemented with minerals. There were no differences in appetite or gain in weight.

In similar experiments sugar was added to the milks to double their caloric value. Again the growth and appetite of the animals were the same on the raw summer and "winter" milks. Pasteurization did not alter significantly the value of the milks, but the summer milk, whether raw or pasteurized, was superior to the pasteurized "winter" milk.

Guinea pigs receiving the raw mineralized milks alone or with sugar died within a comparatively short time. There was no difference in growth performance or time of survival between summer and "winter" milk groups.

The authors state that these experiments give no indication of the presence of a specific new appetite or growth factor in pasture milk.

S.T.C.

#### 420. Influence of Age of Cow on Ascorbic Acid Content of Certified Milk.

A. D. HOLMES AND FRANCIS TRIPP, Research Labs., The E. I. Patch Co., Boston; E. A. WOELFFER, H. P. Hood and Sons, Boston; AND G. H. SATTERFIELD, Univ. of North Carolina, Raleigh, N. C. Food Research, 5: 3, p. 263. May-June 1940.

As a result of the analysis of 659 samples of Guernsey and Holstein milk from animals stall fed on a variety of hays and concentrates, where the ages of the cows ranged from four to eleven years, no consistent relationship was noted between the age of a cow and the amount of ascorbic acid in the milk.

F.J.D.

### ICE CREAM

#### 421. Sugar in Ice Cream. P. H. TRACY, Univ. of Illinois. Ice Cream Field, 35: 5, 68. May 1940.

It is stated that approximately 200,000,000 pounds of sugar are used in the ice cream industry annually. The necessity of using sugar to insure palatability is emphasized and the influence of sugar upon freezing point lowering of ice cream mixes is indicated.

Mention is made of certain products, such as corn syrup or dried corn syrup which have recently been used in the ice cream industry. It is reported that experiments conducted by the author show that high conversion

corn syrup can be successfully used to replace from 50-75 per cent of sucrose in the manufacture of sweetened condensed milk. It is also stated that one-third to one-half the sucrose in ices or sherbets can be replaced by "Sweetose," this new corn syrup which was used in the experiments conducted by the author. It is claimed that this product improved the body and texture of ices and sherbets and enhanced certain fruit flavors, especially pineapple.

In ice cream when replacing 25 to 33½ per cent of sucrose with "Sweetose" on a sweetening value basis of two-thirds, no effect was noticed on mix color, pH or viscosity but the mixes whipped slightly slower. No deleterious effects were noted so far as flavor was concerned, but the body was improved according to the author.

W.C.C.

**422. Vegetable Ice Cream.** VINCENT M. RABUFFO, Editor, Ice Cream Trade Journal. Ice Cream Trade J., 36: 6, 12. June 1940.

Vegetable flavored ice cream has been developed and introduced by Philip Wenger, Tortoni Ice Cream Company, Newark, N. J. Tomato sherbet and spinach, carrot and fresh asparagus ice cream are among those sold.

W.H.M.

**423. Soda Fountain Retail Ice Cream Stores.** W. L. MOLLOY, Sales Mgr., Grand Rapids Cabinet Co. Ice Cream Field, 35: 5, 46. May 1940.

The wide range of conditions under which soda fountains are operated is pointed out. It is claimed that the deciding factor to consider when attempting to determine whether or not sandwiches, lunches, etc. should be served at the soda fountain is whether their introduction will increase the consumption of ice cream and other dairy products sold at the fountain.

W.C.C.

**424. Texture in Ice Cream.** J. H. ERB, Ohio State Univ. Ice Cream Field, 35: 6, 32. June 1940.

The author emphasizes the importance of low temperature drawing of ice cream from the freezer and rapid hardening as a means of improving ice cream texture. He claims that stiff ice cream from the freezer when hardened slowly at 0° F. was about equal in texture to soft ice cream from the freezer if the latter was hardened rapidly.

It is claimed that the ice cream from a continuous freezer compared to ice cream drawn at the same temperature from a batch freezer has a better texture because of the finer incorporation of air in the continuous freezer.

W.C.C.

**425. Plant Maintenance.** F. C. VOGT, Pres., Vogt's Ice Cream Co., New York, N. Y. Ice Cream Field, 35: 5, 50. May 1940.

According to the author each plant has its own specific maintenance

problems. This is especially true with refrigeration systems it is claimed; often lack of flexibility in such equipment makes the problem acute.

With other equipment, maintenance need not be difficult it is stated, since troubles can usually be forecast and proper repairs made before maintenance reaches the acute stage.

The necessity of properly grinding the valves in homogenizing equipment, of carefully handling sanitary piping, of maintaining proper strengths of cleaning solutions through regular and frequent checks, of operating the boiler and other pieces of equipment according to established practices are all mentioned as part of an efficient management program. W.C.C.

**426. Charting Better Retail Store Control.** ANONYMOUS. Ice Cream Field, 35: 5, 29. May 1940.

It is claimed that analyses of many retail stores show lack of adequate and accurate control of merchandise and costs which is often a primary cause of failure. Two charts used by one successful ice cream company are reproduced to illustrate devices that can be helpful. These charts are entitled (1) "Efficiency Rating of Sales People," and (2) "These are Money Losses." They illustrate many points worthy of consideration. W.C.C.

**427. Retail Store Organization.** CHARLES PAYNE, United Farmers Dairy Stores, Inc., Charlestown, Mass. Ice Cream Field, 35: 5, 36. May 1940.

Proper organization according to the author divides the whole job into parts in such a way as to accomplish the desired results. Further, it defines each job as well as the inter-relationship between jobs thereby removing many causes of friction.

Merely assigning certain duties to each employee is not enough. It is claimed that organization necessitates that one (1) analyze or study the work to be done, (2) organize or group the various things to be done, (3) deputize or select proper personnel, (4) train each employee for his job, and (5) supervise or see that the program outlined is accomplished. W.C.C.

**428. Mechanical Refrigeration in Ice Cream Truck Bodies.** P. FORTNEY, Wärtsilä-Fortney Body Co., Cleveland, Ohio. Ice Cream Field, 35: 5, 44. May 1940.

It is claimed that the most widely used type of mechanical refrigeration in the ice cream industry is the hold over system. This system consists of an evaporator coil which is immersed in or adjacent to a eutectic solution of silica jell. The compressor may be on the body or a remote installation. It is claimed that most buyers prefer the compressor on the truck body unless an ammonia compressor is used as the source of refrigeration.

This system according to the author maintains more uniform temperatures in the truck body than other types of refrigeration systems used. The cost of operating the truck bodies is no greater with the load in the truck than with it empty, hence it is common practice not to remove the return load where this system is used. The following operating costs for this system are given by the author. Depending upon power rates it costs from 15 cents to 50 cents per day with an average of 30 cents for a 500 to 600 gallon body. Daily depreciation amounts to 50 cents and service charge 10 cents per day or approximately 90 cents per day total cost.

It is stated that ice cream transport trucks sometimes used the above system of refrigeration but often resort to the constantly operating type, where a generator is mounted on the truck and driven by a power take-off from the transmission. Either direct expansion or partial hold-over coils are used with this system and it is claimed that best results are obtained where partial hold-over coils are provided.

W.C.C.

**429. Refrigeration in the Dairy Industry.** R. A. BRODESSER, Southern Dairies, Inc., Washington, D. C. *Ice Cream Field*, 35: 6, 12. June 1940.

Refrigeration is the most important factor to be considered in the food industry and the machinery required to produce it makes up the largest investment, it is claimed. Multi-stage compressors of the booster type and high speed refrigeration machinery are more efficient than the older types the author states.

It is claimed that a multi-process dairy plant, *i.e.*, one that produces ice cream, milk and other products needs three suction pressures, *viz.*—5, 20 and 30. Multiple header and booster systems make it possible to obtain any one of these pressures. The applications of each of these systems is then briefly discussed. The author refers to a system of refrigeration pipe arrangement as the "bin type hardening room," that his concern has used successfully. He claims that they prefer it to the low temperature blower system.

Referring to refrigerated trucks he claims that hold-over coils and dry ice are both giving satisfactory results.

Important steps in the development of ice cream cabinets are outlined. The trend has been towards a compact, light cabinet of sturdy construction, one which after installation will require the minimum of service. It is also claimed that in areas where the cost of dry ice equals that of purchased power, electrically operated compressors will be obsolete.

W.C.C.

**430. Creating Gallonage.** A. W. SMITH, United Dairy, Springfield, Mass. *Ice Cream Trade J.*, 36: 5, 64. May 1940.

The United Dairy System, Inc., Springfield, Massachusetts, has hit upon a novel way for increasing the sale of ice cream to church socials, picnics,

weddings and parties. It will furnish a portable fountain without rental to any group sponsoring a gathering of any kind. W.H.M.

- 431. The Use of Sugars in Ice Cream.** B. I. MASUROVSKY. *Ice Cream Trade J.*, 36: 5, 69. May 1940.

The author of this article has abstracted an original article entitled, "How One Sugar Compares with Another," by Dr. Stroud Jordan, Food Industries, volume 12, number 3, using sucrose as a standard and having a sweetening value of 100, other sugars have a sweetening value as follows: levulose—175; dextrose—66; corn syrup—30. The caloric value of sucrose is given as 1794 per pound, anhydrous dextrose, 1704 per pound, and hydrated dextrose, 1549 per pound. W.H.M.

- 432. Consumer Educational Trends.** RACHAEL L. REED, Kansas City Dairy Council, Kansas City, Mo. *Ice Cream Rev.*, 23: 7, 39. Feb. 1940.

The ice cream industry stands to gain by cooperating with consumer groups. The reasons for a consumer movement and a brief history of it are given. It is suggested that ice cream manufacturers hold consumer leader conferences, arrange for plant visits, present industrial displays, etc.

J.H.E.

- 433. Efficient Personnel.—No. 1 Problem of the Retail Ice Cream Store Operator.** EDWARD THOM. *Ice Cream Rev.*, 23: 7, 30. Feb. 1940.

This is a portrayal of the policies and plan of operation of the 23 retail ice cream stores. The most important factor in successful operation is personnel. To train efficient managers and clerks and hold them is an everlasting job. An excellent means of building employee morale and bringing about a closer relationship between employer and employee has been a weekly news letter from the manager addressed to all supervisors, store managers, plant superintendents, and owners. J.H.E.

- 434. Better Merchandising Through Drug Store Fountains.** M. A. NEWTON, Wendt's Cream Top Dairy, Niagara Falls, N. Y. *Ice Cream Rev.*, 23: 8, 35. March 1940.

Ice cream manufacturers should aggressively influence druggists to do a better job of merchandising ice cream. Old time fountain arrangements should be modernized. Too often the drug store fountain is unattended while the average store should have an attendant behind it at all times just as special retail ice cream stores have. J.H.E.

- 435. A Method for the Accurate Sampling of Ice Cream.** A. C. MAACK AND P. H. TRACY, Univ. of Illinois, Urbana, Ill. *Ice Cream Rev.*, 23: 8, 36. March 1940.

There has been difficulty in getting a representative fat and solids test in fruit and nut ice cream due to the pieces of flavor material in the melted ice cream. Some testers merely strain out the added material and run the test on the remaining mix. This does not give accurate information even on the unflavored mix. It would be desirable to break up the material fine enough in order to get a representative sample.

An accurate method of sampling and testing fruit ice cream is described. The fruit in a four or five ounce sample of ice cream is thoroughly broken up by means of a malted milk mixer. Tests indicate an accurate test of the product can be made. Nut ice creams tend to show a considerable increase in fat content when the nuts are first broken up by the mixer. This is said to be due to the inclusion of the oil in the nuts. J.H.E.

- 436. Sugars in Ice Cream.** R. J. TREBILCOCK, Corn Products Sales Co., New York. *Ice Cream Rev.*, 23: 8, 42. March 1940.

This is a review of the function of sugar in ice cream and a discussion of the various types of sugar. When dextrose is used to replace 25 per cent of the sucrose, the freezing point is lowered .65 of a degree F. J.H.E.

- 437. Handling Seasonal Changes of Labor Requirements in an Ice Cream Plant.** I. R. KRILL, Moores and Ross, Inc., Columbus, O. *Ice Cream Rev.*, 23: 8, 38. March 1940.

A plan is described for handling the problem of seasonal employment in the ice cream industry. Meeting these requirements successfully involves three things: first, carefully selecting workers who have good health and are reliable; second, integrating the labor of the new employees with that of the old employees in such a way as to maintain efficient production and avoid waste; and third, managing by wise foresight and a few simple precautions to keep at a minimum the expense of unemployment compensation and industrial insurance.

The best market for summer labor is in two groups: first, women and girls who are seasonally employed in winter by department stores or seasonal industries; and second, students studying dairy manufacturing, who in the dull season of the ice cream business are attending school. J.H.E.

- 438. Soliciting Consumers by Mail.** ANONYMOUS. *Ice Cream Rev.*, 23: 11, 23. June 1940.

A mail solicitation plan for reaching ice cream consumers is described. The experience thus far has been successful in gaining new customers.

J.H.E.

- 439. Serum Solids for Ice Cream.** H. E. OTTING, M & R Dietetic Labs., Columbus, O. *Ice Cream Rev.*, 23: 9, 37. April 1940.

Several types of milk solids are available for ice cream. The kind used should depend on: (1) freedom from heated or cooked flavors; (2) stability or keeping quality; (3) laws of boards of health governing territory of distribution; (4) water absorbing ability; (5) composition of mix; (6) cost; (7) availability. The forewarning temperature, degree of concentration and characteristics of each type of serum solid source is discussed. J.H.E.

- 440. What is Adequate Pasteurization of Ice Cream Mix.** T. V. ARMSTRONG, Ohio State Univ., Columbus, O. *Ice Cream Rev.*, 23: 9, 41. April 1940.

This article is a review of previous work on the thermal death points of pathogens in ice cream. The recent work on the *Escherichia-aerobacter* group of bacteria and the phosphatase test is considered. This leads the author to conclude that 150° F. for 30 minutes should be the minimum for adequate pasteurization. He also cites that the recommendations of the Committee on Ice Cream Sanitation of the International Association of Milk Sanitarians are 155° F. or higher for 30 minutes, or 180° F. or higher for 16 seconds as the short time method.

J.H.E.

- 441. How to Wash Ice Cream Equipment.** W. B. COMBS, Univ. of Minnesota, St. Paul, Minn. *Ice Cream Rev.*, 23: 9, 52. April 1940.

Equipment should first be rinsed with warm water and then dismantled. Prepare washing solution by adding washing powder to a pail of water and dissolve completely before use. Use a stiff brush to scrub all parts of equipment. Finally rinse with warm water and steam with a steam hose. The cleaning of special equipment is outlined. For metal equipment use to each 50 gallons of water a washing powder in the following amounts: 1 lb. neutral soda,  $\frac{1}{2}$  lb. soda ash, and  $\frac{1}{2}$  lb. trisodium phosphate. All equipment should dry quickly after cleaning. A chamois skin is recommended for polishing outside surfaces.

J.H.E.

- 442. How to Sterilize Equipment.** H. Macy, Univ. of Minnesota, St. Paul, Minn. *Ice Cream Rev.*, 23: 9, 54. April 1940.

This is an excellent article giving detailed instruction for sterilizing each piece of equipment commonly found in an ice cream plant. The system is rigid. In general, the equipment should be steamed until the condensate at the outlet of the equipment is above 180° F. for at least three minutes. Assembled equipment may be rinsed with chlorine solution just before use. This should contain 50-200 parts per million of available chlorine, depending upon the period of exposure. The author is of the opinion that a fresh water rinse is desirable following chlorine sterilization.

J.H.E.

- 443. New Competition for the Ice Cream Manufacturer.** H. H. SOMMER, Univ. of Wisconsin, Madison, Wis. Ice Cream Rev., 23: 9, 94. April 1940.

Ice cream mixes and mix products for home use are on the market and are being tried by many house-wives. The type of products that have been placed on the market may be classified as follows: (1) mix bases consisting of stabilizer, sugar and flavors; (2) mix bases similar to number one but containing some skimmilk solids; (3) powdered ice cream mixes; (4) canned, sterilized ice cream mixes; (5) bottled, unsterilized, ice cream mixes. Some of these products involve difficulties with respect to mix composition, mix processing, freezing and whipping. Ice cream mixes, specially designed for home freezing and delivered freshly bottled on milk routes, at present are on the increase. Similarly, canned ice cream mixes have possibilities but also have some disadvantages, so the future of these products is difficult to predict. The belief is expressed that none of these products afford any real economy to the housekeeper but pride in her own handiwork may make for a permanent establishment of these home ice cream mixes.

J.H.E.

- 444. Reduce Power Costs.** ANONYMOUS. Ice Cream Rev., 23: 10, 38. May 1940.

Operating in a community where purchased electric power cost  $3\frac{1}{4}$  cents per kilowatt, a California ice cream manufacturer installed a new Diesel engine for generating his own current. After the experience of operating 8,000 hours, the owner concludes that his electricity now costs him less than one cent per kilowatt.

J.H.E.

- 445. Keeping down Bacteria Counts.** WESLEY SCHWEN, Schwen's Ice Cream Co., Blue Earth, Minn. Ice Cream Rev., 23: 10, 40. May 1940.

This is a detailed explanation of how one ice cream plant practices good sanitation. Bacterial counts are run on all mixes and frequent line run tests and *E. coli* tests are used to determine source of contamination. Many helpful operating suggestions are given.

J.H.E.

- 446. Pan Condensed Ice Cream Mixes.** R. A. LARSON, Michigan State College, East Lansing, Mich. Ice Cream Rev., 23: 10, 34. May 1940.

Baumé hydrometer readings were determined for a number of ice cream mixes made in the vacuum pan. For all the mixes studied within the range of 115–155° F. a 5° F. change in temperature caused a .2° Baumé change. Charts are given showing proper Baumé readings at various temperatures for 12 different mixes. The directions are given for predicting the correct Baumé reading of any composition mix.

- 447. Proper Paint in the Ice Cream Factory.** J. W. THOMPSON, Pittsburgh Plate Glass Co., Milwaukee, Wis. *Ice Cream Rev.*, 23: 10, 54. May 1940.

This is an informative article giving much up-to-date information on the subject of proper paint for the walls, equipment and floors of dairy plants. Fungicide paints, fume resisting enamels, paint odor contamination, synthetic resins, the painting of refrigeration lines, and use of color and lighting are all discussed.

J.H.E.

- 448. Proper Accounting in the Ice Cream Business.** EDWIN STOVALL. *Ice Cream Rev.*, 23: 11, 33. June 1940.

This is a plea for simplification of accounting systems so that essentials are plainly and accurately portrayed.

J.H.E.

- 449. Some Points to Consider before Beginning Distribution of Frosted Foods.** RUSSEL BROWN, Birds Eye Frosted Foods Co. *Ice Cream Rev.*, 23: 11, 38. June 1940.

Ice cream manufacturers are said to handle 18½ per cent of all frosted food sold. This article is a discussion of a number of precautions to consider before venturing into the business.

J.H.E.

- 450. Point-of-Sale—Sanitation.** H. T. SMITH. *Ice Cream Trade J.* 36: 6, 8. June 1940.

The use of paper cups and other single service containers have helped many ice cream dealers in solving the dishwashing problem and made it possible for them to comply with the stricter sanitary rules which are now in force in many cities.

W.H.M.

- 451. Kansas Ice Cream Survey.** H. E. DODGE. *Ice Cream Trade J.* 36: 6, 36. June 1940.

The results of the annual ice cream surveys conducted by the Dairy Division of the Kansas State Board of Agriculture and the Dairy Department of the Kansas State College, show a steady improvement in the quality of the ice cream as indicated by the bacterial counts. The number of counter freezers increased from 62 in 1935 to 203 in 1938, followed by a decline to 202 in 1939. The average yearly gallonage was 3,000 gallons for the counter freezers and 28,000 gallons for the wholesale manufacturers.

W.H.M.

- 452. Early History of the Ice Cream Industry.** W. H. LIST, JR., Sec'y, Pa. and N. J. Assn. of Ice Cream Mfgs. *Ice Cream Trade J.*, 36: 5, 12. May 1940.

This article is very enlightening to those interested in the history of the ice cream industry. Pictures are presented and description given of the various developments which have taken place down through the years.

Starting with the introduction of the wholesale ice cream business in 1851 by Mr. Fussell in Baltimore, the story relates the experiences of many of the pioneers of the industry. Sanitation, source of supply, distribution, prices and regulations were problems which faced the ice cream manufacturers at the start of the twentieth century. Pasteurization, and homogenization of ice cream mix started about 1904. Federal pure food laws were passed in 1906 followed shortly thereafter by laws in several states. The brine freezer was introduced in 1902, the direct expansion in 1914 and the fore-runner of the present continuous freezer in 1928. Not until 1906 was electricity used as power for ice cream freezing. Uniform cost accounting was introduced about 15 years ago. Trucks were used for delivering of ice cream for the first time in 1907, and in 1925 the first mechanically refrigerated trucks were put into operation. Mechanically refrigerated ice cream cabinets were developed in 1920, and six years later dry ice was used as a refrigerant for ice cream. Paper ice cream cans made their appearance about 10 years ago followed by many types of paper ice cream containers. Ice cream associations and state universities have played an important role in the development of the ice cream industry.

W.H.M.

- 453. Gallonage.** VINCENT M. RABUFFO, Editor, *Ice Cream Trade J.* *Ice Cream Trade J.*, 36: 5, 16. May 1940.

The story of the development and operation of the Philadelphia Dairy Products Company, one of the nation's largest ice cream plants with an annual production of 5,000,000 gallons is described in this article. W.H.M.

- 454. The Beginning of the Wholesale Ice Cream Business—1851.** M. T. FUSSELL. *Ice Cream Trade J.*, 36: 5, 37. May 1940.

This article relates the story of the beginning of the wholesale ice cream business by Jacob Fussell in Baltimore in 1851. The operation of the Baltimore plant was followed by one in Washington, D. C., 1856; Boston, 1862, and New York in 1864. W.H.M.

- 455. Ice Box Competition.** HOWARD YAW. *Ice Cream Trade J.*, 36: 5, 28. May 1940.

Statistics are presented showing the marked increase in the sale of bottled carbonated beverages and fruit juices. The writer states that as the sale of these products for home consumption has increased there is evidence of a decline in the consumption of ice cream in the home. W.H.M.

- 456. Homogenization, a Comparison of Pressure and Rotary Type Machines.** C. D. DAHLE, Pennsylvania State College, AND C. M. Moss, Dairymen's League. *Ice Cream Trade J.*, 36: 6, 18. June 1940.

A gear type and eccentric type of rotary homogenizer for the homogeni-

zation of ice cream mix were compared with a pressure type. The author states that all machines gave satisfactory results from the standpoint of body, and texture of the ice cream, fat globule size overrun, and fat clumping. There was some difference in the rotary machines from the standpoint of pressure fluctuation temperature rise and sanitation and uniformity of operation.

W.H.M.

## MILK

- 457. High Quality Milk Production.** L. E. PARKIN. Pennsylvania State College Cir. 221, 12 pages. 1940.

This circular is for the producer of milk. It considers the essentials in the production of high quality milk involving the attendants, cows, environment, feed flavors, milking equipment and methods of milking and handling the milk.

W.E.P.

- 458. Some Legislative Aspects of Chocolate Milk Distribution.** GIDEON HADARY. Milk Dealer, 29: 8, 78-82. May 1940.

A brief discussion of the laws and regulations governing the production and sale of chocolate milk. In summarizing his discussion the author states that one can regard chocolate milk primarily as: (1) an outlet for the sale of whole milk, overlooking the fact that it is a beverage; (2) a beverage, overlooking the fact that it contains milk; (3) a beverage containing milk.

The first two approaches are wrong. The first approach is that taken by the courts in Florida, while the second is taken by the State of Kentucky. The third approach, the one that is the best, takes into consideration that the drink is an outlet for milk sales; yet, the fact that it is a beverage superior to others by the presence of milk in it is not overlooked. Wise municipal regulation will set this approach as criteria in establishing chocolate milk legislation.

C.J.B.

- 459. What We Know about Homogenized Milk.** F. J. DOAN, Pennsylvania State Collgee, State College, Pa. Milk Dealer, 29: 8, 42-52. May 1940.

The advantages, disadvantages, properties and characteristics and the problems in the production and distribution of homogenized milk are discussed. It is also pointed out that where only a medium efficiency of homogenization is required, the rotary homogenizer will give as good results as the piston; but if high efficiency of homogenization is needed, then the piston machine will usually give superior results.

C.J.B.

- 460. Control of Flavor in Milk Heated to High Temperature.** I. A. GOULD, Dept. of Dairying, Michigan State College, East Lansing, Mich. Milk Dealer, 29: 8, 70-76. May 1940.

Report of a study to determine the possibility of using a combination of

both copper and homogenization on high-temperature treated milk to control the cooked and oxidized flavor. The author summarizes his work as follows:

Milk heated to 180° F. and then treated with small quantities of copper salts will lose its cooked flavor and become oxidized. However, if the milk is homogenized either before or after the addition of copper, the oxidation is prevented and the milk in time will assume a normal flavor.

Milk to which was added 1.5 to 2 p.p.m. of copper and which was then homogenized, showed practically no cooked flavor after 24 hours of storage, and the milk was fine-flavored even after 120 hours. When less copper was used, the cooked flavor disappeared somewhat more slowly. The cooked flavor disappeared slightly more rapidly if the copper was added at 145° F. than when added at 180° F. Somewhat similar results were secured when the copper was added after homogenization.

The data presented herein offer a practical application of previous findings dealing with the cooked flavor. The results show that it is possible to secure an excellent, normal-flavored milk even though the milk has been subjected to temperatures sufficiently high to cause a strong cooked flavor to appear. The combination of copper salts with homogenization in a proper manner might be used under certain conditions to control milk flavors of highly heated milk.

Proper use of the findings of this paper would permit an operator to prepare a milk with low curd tension, as brought about by heat and homogenization without the disagreeable off-flavor which such milk usually possesses. It is realized, however, that application of these findings in a commercial manner must be done with the approval of health officials.

C.J.B.

## PHYSIOLOGY

- 461. Destruction of Ascorbic Acid in the Rumen of the Dairy Cow.** C. A. KNIGHT, R. A. DUTCHER, N. B. GUERRANT AND S. I. BECHDEL, Departments of Agricultural and Biological Chemistry and Dairy Husbandry, Pennsylvania State College. Proc. Soc. Exp. Biol. and Med., 44: 90, 1940.

Neither the feeding of 100 grams and 150 grams of synthetic ascorbic acid mixed with corn silage nor the placing of 100 grams of ascorbic acid directly in the rumen through a fistula opening increased the ascorbic acid values of the blood plasma and of the milk when compared with those values obtained while the cow was on a standard ration unsupplemented with the vitamin. A slight increase was noticed in the amount of ascorbic acid found in the 24-hour sample of urine for the periods during which the vitamin was administered. A rapid and pronounced destruction of ascorbic acid in

the rumen was demonstrated by removal and analysis of samples of the rumen contents at regular intervals after the cow had been fed. Ascorbic acid added to rumen contents in vitro and stored in a dark-glass, stoppered bottle at 39°-42° C. disappeared at much the same rate as that of the in vivo experiments.

R.P.R.

462. **The Effect of Certain Experimental Conditions upon the Thyrotropic Hormone Content of the Albino Rat.** C. W. TURNER AND P. T. CUPPS, Department of Dairy Husbandry, University of Missouri. *Endocrinology*, 26: 1042, 1940.

A study of the thyrotropic hormone content of the pituitaries of albino rats of both sexes, weighing between 150 and 200 gm., following various periods of gonadectomy was reported. Following gonadectomy, the thyrotropin content of the A P of both males and females was reduced slightly after 20 days and rather markedly after 66 days. Replacement therapy with estrogen at the rate of 40 r. u. and androgen at the rate of 200 gamma daily appeared to maintain the normal level of thyrotropin in the castrate female but not in the male. Thyroidectomy of male rats for periods of 40 days and 6 months resulted in a reduction of about 50 per cent in the thyrotropin content of the A P. In contrast, females similarly treated maintained their normal content or showed a slightly increased level of hormone. R.P.R.

463. **The Comparative Assay of Gonadotropic Substances on Rats, Mice and Chicks.** JOHN S. EVANS, LEONARD HINES, ROGER VARNEY AND F. C. KOCH, Dept. of Biochemistry, University of Chicago. *Endocrinology*, 26: 1005, 1940.

In the assay of unfractionated pituitary extracts, the mouse uterus was about 66 times as sensitive as the rat ovary, and about 10 times as sensitive as the chick testes. In the assay of pregnant mare serum preparation (gonadogen), the mouse uterus was about 60 times as sensitive as the rat ovary, and about 90 times as sensitive as the chick testes. In the assay of normal male urine preparation (prospermin), the mouse uterus was about 90 times as sensitive as the rat ovary, and about 55 times as sensitive as the rat uterus. The response of the chick testes to normal male urine was doubtful. The mouse uterus was about 30 times as sensitive as the rat ovary to menopause urine preparation (gamone) and about 6 times as sensitive as the rat uterus. The response of the chick testes to menopause urine was doubtful. R.P.R.

464. **Utilization of the Ketone Bodies in Normal Animals and in Those With Ketosis.** RICHARD H. BARNES, D. R. DRURY, P. O. GREELEY AND A. N. WICK. Department of Physiology, School of Medicine,

University of Southern California, and Scripps Metabolic Clinic.  
Amer. Jour. Physiol. 130: 144-150. 1940.

The authors suggest that the production of ketone bodies by the liver and utilization of them by the other tissues is an important, though not necessarily inevitable, route for the catabolism of fatty acids. When the organism is in a state of ketosis, increases in metabolic rate (as in exercise) probably increases the rates of production and utilization of these substances.

The results indicate that from 30 to 80 per cent of the energy requirements of the tissues (rabbits, goats, dogs) in ketogenic states may be supplied by combustion of ketone bodies.

The authors believe that more than one molecule, possibly four, of ketone bodies may be formed per molecule of fatty acid catabolized. D.E.

**465. Induction of Lactation in Goats with Diethylstilboestrol Dipropionate.** S. J. FOLLEY, HELEN M. SCOTT WATSON AND A. C. BOT-TOMLEY. National Institute for Research in Dairying, Reading. Proc. Physiol. Soc., Jour. Physiol. 98: 15-16. 1940.

One gram of one per cent diethylstilboestrol dipropionate ointment was applied three times a week to the udders of three virgin female goats and daily milking begun. After a latent period of 30 days during which a few ml. of fluid were secreted daily, there was a sudden increase in milk yield to a maximum of 1500 ml. daily and then a slow decline. The milk secreted was normal and the milk production curve resembled a normal lactation curve. The results with a virgin heifer were disappointing, the secretion never passing the colostral stage. These experiments indicate that oestrogens may not inhibit lactation in ruminants but, at least when injected into goats in limited amounts, will cause udder development and copious secretion of normal milk without need for prolactin treatment. D.E.

**466. Glycogen and Calcification.** G. E. GLOCK. Dept. of Physiology, Bedford College, University of London. Jour. Physiol. 98: 1-11. 1940.

It is suggested that in both tooth and bone development, the glycogen of the bones might first initiate the differentiation and later serve as a primary source of the phosphoric esters required for calcification. If this is true the inhibitory effect of NaF on bone calcification might be attributed to the low glycogen content which resulted from the administration of flourine.

D.E.

**467. The Partition of Serum Calcium about the Time of Parturition in the Dairy Cow.** J. DUCKWORTH AND W. GODDEN. The Rowett Institute, Aberdeen, Scotland. J. Dairy Res. 11: 9-14. 1940.

Data are given of the variations in the calcium ion, ultrafiltrable calcium

complex, non-ultrafiltrable calcium complex and protein-bound calcium at normal parturition in the dairy cow. All the values found for total serum calcium were higher a few days before calving than those found either a few weeks before or after calving. At the time of actual calving, however, there was generally a reduction of about 10 per cent in the total calcium.

The values obtained for calcium ion ( $\text{Ca}^{++}$ ) were generally, but not always, maximal at or near the time of calving.

The values for the ultrafiltrable calcium complex and for non-ultrafiltrable calcium complex all showed a reduction at the time of calving.

The values for protein-bound calcium were generally, but not invariably, increased near the time of calving.

S.T.C.

#### **468. A Long-Term Study of the Partition of Serum Calcium in Ayrshire Cows.**

W. GODDEN AND J. DUCKWORTH. The Rowett Institute, Aberdeen, Scotland. *J. Dairy Res.*, 11: 15-21. 1940.

The following average fractionation of serum calcium in the dairy cow were reported:

Calcium ion ( $\text{Ca}^{++}$ )	10-12 per cent.
Ultrafiltrable calcium complex	40-45 per cent.
Non-ultrafiltrable calcium complex	about 20 per cent.
Protein-bound calcium	about 25 per cent.
Ultrafiltrable calcium	50-53 per cent.

S.T.C.

#### MISCELLANEOUS

##### **469. Cold Storage Locker Operation.** ANONYMOUS. *Ice Cream Rev.*, 23: 11, 40. June 1940.

This is a brief report of the second annual cold storage locker operators' conference at the University of Wisconsin, April 30 to May 1, 1940. Suggestions for handling frozen meat products are given.

J.H.E.

##### **470. What Shall I Use for Fuel in the Dairy Plant?** S. KONZO, Eng. Exp. Sta., Univ. of Illinois, Urbana, Ill. *The Dairy World*, 19: 2, 16. July 1940.

The author briefly summarizes the advantages of coal (hand and stoker fired), oil and gas as boiler fuels on a comparative basis. He gives the costs of the three types of fuel, under stated conditions, as: 3.8 cents for coal, 4.9 cents for oil and 7.5 cents for gas, per 100,000 B.T.U. From the data presented costs under other price conditions can be readily calculated. Methods by which maximum combustion efficiency may be obtained are presented.

F.J.D.

- 471. Making Figures Effective to Management.** RAY C. PERKINS, Adohr Milk Farms, Los Angeles, Calif. The Assn. Bull. Intern. Assn. Milk Dealers. 32nd year. No. 15: 341-346. March 1940.

To be effective, reports should be brief, concise, definite and imaginative. The results of all branch plants should be included on each report for comparative purposes. Present to management in permanent typed form only the reports that carry an effective message. Seven such reports are described.

E.F.G.

- 472. Problems of Internal Audit Control.** PAUL L. SCOTT, Bordens Delivery Co., San Francisco, Calif. The Assn. Bull. Intern. Assn. Milk Dealers. 32nd year. No. 15: 347-352. March 1940.

One purpose of internal audit control is to remove the temptation for the employee stealing property or money by involving at least three persons in a suitable system of control. The essential details of such a system are outlined. An effective plan of internal control should cover everyone from routeman to president and be sufficiently rigid so that each one will recognize the futility of any criminal "intention" in handling company transactions. An extensive list of references in connection with internal control operation and internal auditing is appended.

E.F.G.

- 473. Approaches to Budgetary Control through Planned Performance.** ANSON HERRICK, C.P.A. The Assn. Bull. Intern. Assn. Milk Dealers. 32nd year. No. 15: 353-362. March 1940.

The characteristics of budgets in industry for different purposes and different operations are given. It is also explained how these budgets may be used to control costs and operations. In situations where production is not uniform the unit cost basis must be used. It is believed that standard cost rates are adaptable in the majority of dairy manufacturing processes and are at least the most convenient and economical basis for using planned performance and costs as efficiency standards for the controlling of actual production costs.

E.F.G.

- 474. Is Collective Bargaining the Answer to our Labor Relations Problems?** ALMON E. ROTH, President, San Francisco Employers' Council. The Assn. Bull. Intern. Assn. of Milk Dealers. 32nd year. No. 16: 367-382. May 1940.

Strikes and threats of strikes have far reaching economic consequences in redistribution of markets. Many concrete instances are given. The author addresses himself to the question: What are the chances of establishing peaceful labor relations through collective bargaining? The many essential factors in orderly collective bargaining are discussed together with unsolved difficulties. Conceding a conflict of interests between employer

and employee the author states that the most that can be hoped for is to develop a fair and honest attitude on the part of each of the parties toward the other and to insist that peaceful means be developed for settling disputes without costly interruptions or unnecessary hardships. E.F.G.

- 475. Prevention of Fly Contamination on the Farm.** L. J. Hois, Geuder, Paeschke & Frey Co., Milwaukee, Wis. Milk Dealer, 29: 8, 66-68. May 1940.

The use of strainer covers and screened racks for milk pails and cans is recommended as a means of fly control. This localized control is in addition to the usual methods of fly control such as destroying or treating feeding and breeding places. C.J.B.

- 476. Tackling the Distribution Problems of the Dairy Industry.** L. R. SCAFFE, White Motor Co., Cleveland, Ohio. Milk Dealer, 29: 8, pp. 34-35, 82-84. May 1940.

The distribution problems of the dairy industry are discussed mainly from the transportation standpoint. The author states that: "In the field of distribution lies our greatest future opportunity of cutting costs and increasing profits." C.J.B.

- 477. Proper Paint in the Dairy Plant.** J. W. THOMSON, Pittsburgh Plate Glass Co., Milwaukee, Wis. Milk Dealer, 29: 8, pp. 32-33, 61-64. May 1940.

A discussion of the proper paint to use in different sections of a dairy plant. C.J.B.

- 478. Water Supply vs. Quality in the Dairy and Ice Cream Plant.** M. E. PARKER, Beatrice Creamery, Chicago, Ill. Ice Cream Trade J., 36: 5, 31. May 1940.

The importance of the water supply in the production of quality dairy products is discussed. Since water is an ingredient used in the manufacture of sherbets, ices, fruit juice drinks and other dairy products, it should be free from sediment and objectional odors which might be imparted to the finished product. By tasting and smelling water which has been heated to 100° to 120° F. the operator can usually detect obvious defects.

The chemical character of the water used for cleaning purposes may be responsible for milk stone accumulation on equipment unless the proper cleaner is selected.

Metallic and other off flavors have occurred in dairy products contaminated with water or steam which contained impurities. The use of steam separators to remove impurities and the chlorination of wash water have been effective measures in preventing off flavor from infected water.

W.H.M.

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## ABSTRACTS OF LITERATURE

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Columbus, Ohio

## MILK AND MILK PRODUCTS

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International Association of Milk Dealers	The Royal Technical College, Copenhagen, Denmark
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New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

### ADVANCE ABSTRACTS OF REPORTS TO APPEAR IN THE JOURNAL OF DAIRY SCIENCE

- 479. Official Body and Texture Criticisms of Dairy Products Judged in the National Contest.** G. M. TROUT, WILLIAM WHITE, P. A. DOWNS, M. J. MACK, AND E. L. FOUTS, American Dairy Science Association Committee on the Judging of Dairy Products.

A study of the official body and texture criticisms of butter, cheese and ice cream samples used in the Students' National Contest in the Judging of Dairy Products during the fourteen year period from 1926 to 1939 inclusive shows that body defects of butter were encountered but a few times, whereas in cheese and ice cream they were encountered in the greater majority of cases. Predominating body and texture criticisms were: for butter, "leaky"; for cheese, "open," "weak," "pasty" and "mealy"; and for ice cream "coarse" and "weak" for the frozen product, and "curdy" for the melting appearance. Approximately two body and texture criticisms were used for each sample of cheese and ice cream so criticized whereas one was sufficient in the case of butter.

- 480. Preliminary Observations on Chemical Changes of Rumen Ingesta with and without Urea.** M. I. WEGNER, A. N. BOOTH, G. BOHSTEDT, AND E. B. HART. From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

Dry matter, fiber, ether extract, ammonia nitrogen, non-protein nitrogen, and total nitrogen have been determined on rumen contents of a fistula heifer on a basal ration of corn silage, timothy hay, and corn and oats with and without added urea. The level of total nitrogen and fiber found in the rumen material is distinctly higher than in the ration fed. Urea nitrogen or ammonia nitrogen when ingested as 1 to 5 per cent of the dry matter of the ration fed disappeared from the rumen in 4 to 6 hours after feeding. A definite increase in the percentage of protein nitrogen of the rumen ingesta was produced by adding 5 per cent urea to a low nitrogen basal ration.

- 481. The Relation of Mastitis to the Level of Ascorbic Acid and Certain Other Constituents in Milk.** E. P. REINEKE, E. R. GARRISON, AND C. W. TURNER, Missouri Agr. Exp. Sta., Columbia.

The ascorbic acid content of the milk from ninety individual quarter samples from nineteen cows in the University of Missouri dairy herd was correlated with the incidence and severity of mastitis and the level of chlorides in the milk.

In early stages of mastitis the ascorbic acid of the milk was reduced about 10 per cent and in advanced cases it was reduced by as much as 30 to 50 per cent. It was shown that there is a trend toward an inverse relationship between ascorbic acid and chloride in mastitis milk and also in the milk produced following perfusion of the mammary duct system with a hypertonic solution.

Long chain streptococci isolated from mastitis milk were shown to actually retard the rate of oxidation of ascorbic acid *in vitro*. A higher ascorbic acid oxidase content in mastitis milk was also ruled out as a factor causing the reduction in ascorbic acid.

The theory is advanced that the effect of mastitis upon the level of ascorbic acid in milk is exerted indirectly by producing a change in the selective permeability of the milk secreting cells in relation to the osmotic equilibrium existing between blood and milk.

**482. Antioxygenic Fractions of Oat and Soya Bean Flour.** C. D. DAHLE AND D. H. NELSON, Dairy Department, The Pennsylvania State College.

An attempt was made to determine the active fraction of two cereal anti-oxidants—namely, oat and soya bean flour, when used in pure butterfat. Aqueous, acetone, alcohol, ether, and hexane extracts were made. A phospholipid extract was obtained from the flours and studied in this connection.

In the trials studied the phospholipid and alcohol extracts exhibited the greatest anti-oxygenic properties in pure butterfat held at 60° C.

**483. The Riboflavin Content of Milk as Influenced by Diet.** PAUL JOHNSON, L. A. MAYNARD, AND J. K. LOOSLI, Laboratory of Animal Nutrition, Cornell University, Ithaca, N. Y.

Experiments with cows, involving 460 determinations made over a period of 9½ months, showed clearly that the riboflavin content of milk can be influenced only to a limited extent by the diet. When cows were transferred from pasture to a ration of natural feeds selected to be very low in riboflavin its content in the milk decreased about 25 per cent. Increasing the riboflavin intake 30 to 50 per cent by feeding a molasses-yeast by-product caused only a temporary increase in the concentration of riboflavin in the milk. A winter ration consisting of good quality hay, acid grass silage and a grain mixture maintained the milk riboflavin at the pasture level.

Goats fed a riboflavin-free purified diet continued to secrete large amounts of riboflavin in the milk, indicating this factor is not a dietary essential for lactation in the goat. No consistent difference was observed in the milk yield or the riboflavin concentration when the purified diet was supplemented with a molasses-yeast by-product supplying riboflavin. Like-

wise, no advantage in milk secreted was noted between a thiamin-deficient purified diet and one adequate in it and other factors of the B-complex.

The data from both cows and goats indicate that there is an inverse relation between the milk yield and the riboflavin concentration of the milk.

## BOOK REVIEWS

- 484. The Butter Industry.** O. F. HUNZIKER. Third Edition, 1940, 780 pages, illustrated. Published by the Author, La Grange, Ill. Price \$6.50.

Since the publishing of the second edition of this authoritative work in 1927, developments along many lines have taken place in the creamery industry, and Dr. Hunziker brings this comprehensive and exhaustive treatise up-to-date with his usual unequaled thoroughness. New material has increased the volume to 769 pages of text, more than one-fourth larger than the second edition. The organization of the book follows the same general plan.

A new chapter with much new material on steam power, refrigeration and water-tempering systems, is added. A consideration of corrosiveness of refrigerating brines makes this phase of the work more complete, along with a discussion of boilers and feed waters.

An entire chapter is devoted to creamery equipment—materials of construction, mechanical care and use. The sanitary care of the churn, one of the most important phases of sanitation in the production of butter of good bacteriological keeping quality, is given thorough treatment, and the factory man will find such material extremely helpful. The hope of the sanitarian—the all-metal churn—receives attention along with the no-roll wood churning of recent date.

In the discussion of the classification of cream grades the author takes his accustomed strong stand on the necessity for cooperation among all components of the industry in paying for cream on a quality basis in order for progress in cream improvement and quality of butter to occur. A system of grading cream looking toward the establishment of a national brand of American butter is suggested.

The introduction in recent years of steam-injection pasteurization has added new material to the chapter on pasteurization, and the increased importance of fat losses in buttermilk with this system of pasteurization is discussed. Recent developments in the treatment of cream for the removal of objectionable flavors and odors are described and the author draws on his wide experience in this field in evaluating the merit of the various procedures.

The chapter on starter-making and cream ripening is valuable in bringing the reader up-to-date on the essentials of the progress that has been

made in recent years with the recognition of the role of diacetyl and acetyl-methylcarbinol in the production of the desired flavor in butter.

The churning process itself, the focal point of butter-making, is dealt with exhaustively from theoretical as well as practical viewpoints. The pH of butter and its relation to churning acidity of the cream are discussed, as well as the significance of the pH of butter in the determination of keeping quality in cold storage.

The chapter on packaging butter brings this important subject up-to-date. Apparently, foreign countries have gone further with the packaging of butter direct from the churn than the U. S. Undoubtedly, this phase of the butter industry will see further developments in the next few years. The chapter on "Markets and Marketing of Butter" contains new material on the intricacies of trading in "futures," and the new "Official U. S. Standards for Quality of Creamery Butter," effective April 1, 1939, are given in detail.

"Butter Defects" occupies, as a chapter, the prominent position in point of increased size and detail of development that its importance warrants. The student and research worker will find in this chapter a thorough development of the problems of butter quality that face the industry, and in the presentation of unsolved problems, a stimulus to continued and thorough investigation. "Surface taint" and allied defects in flavor of butter, due to bacterial decomposition of the protein, have been subjected to much careful investigational work in recent years. The rise of this type of defect in butter in this country, and the present status of the problem are presented with definite suggestions as to means of prevention. The research worker in bacteriology will find this subject intriguing. New information on defects in body and texture is made available, especially for the prevention of crumbly and sticky body, so commonly found in winter butter in this country.

In format the third edition is patterned after the second and presents a physical appearance that will be a credit to any library. The broad scope of the work, embracing as it does all phases of the butter industry, assures that it will follow its predecessor in an international circulation among practical buttermakers, students, research workers, and officials of law-enforcing agencies.

W. A. CORDES

**485. Applied Mycology and Bacteriology.** L. D. GALLOWAY AND R. BURGESS. Price \$4.00, 186 pages, illustrated. Distributed by Chemical Publishing Co., Inc., 148 Lafayette St., New York, N. Y.

This book presents a brief summary of the recognition, methods of handling, and control of microorganisms of special interest to industry. Due to the breadth of the field treated the discussions necessarily omit detailed information on many of the subjects included. Numerous references,

however, enable the reader to use this book as an introduction to the various fields of applied mycology and bacteriology.

The contents of the book are as follows: Part I. Fungi, bacteria, apparatus and sterilization, isolation and examination of microorganisms, culture media and stains, metabolism of microorganisms, control of microorganisms. Part II. Food industries, fermentation industries, textile industries, hygiene, agricultural applications, miscellaneous.

The practical dairyman and research worker in the dairy field will find only a brief treatment of their subject. Chapters on food industries, fermentation industries and particularly textile industries present very good general discussions.

This book is well written throughout. It should prove useful to the student as a supplementary text and to the industry as a guide to information on the recognition, methods of handling, and control of microorganisms in the food industry.

P.R.E.

**486. Milk Distribution as a Public Utility.** W. P. MORTENSON, Univ. of Wis. July, 1940. 213 pages. Price \$2.50. Published by University of Chicago Press, Chicago, Ill.

The reviewer is desirous of stating at the outset that in the light of its title, this book definitely is not of the Union Square soap-box oratory type. The author has based his discussion of the subject with facts in the study of operating costs of many milk plants, mainly in Wisconsin. Nor is the book a ponderous statistical handbook. It contains a number of selected summarized tables that present the points the author is making.

As a background there is an interesting review of the economic philosophies that have lead to the type of dairy organizations, both large and small, such as we know today. Whether these businesses fall into the category of public utilities is defined by describing what constitutes a public utility. Operating a milk business in a community as a utility means, concisely, consolidating the motions in the business. The author has discussed specifically, the potential savings that would evidently be possible by unifying the operations of the several plants in a municipality. The estimates are derived from the knowledge of what men, machinery and time are doing, and what these factors reasonably can do. The potential efficiencies are cited for the various items of cost, as plant equipment, plant labor, distribution and so forth. Nor are the probabilities of not making savings overlooked in this review. An interesting confirmation of the appraisal of the benefits of consolidation is in part shown by the review in a chapter of the profits of large and small companies for various years.

What are the possibilities of legal sanction of unification of the milk business? The author has presented in a chapter excerpts of cases (principally U. S. Supreme Court) involving other businesses of the same general

nature determining whether they fell into groups having great liability to public interest. The Supreme Court has not ruled on the legality of the exclusive-franchise for operation of a unified milk business. There are indicated the legal precedences which might make such regulatory action possible, and the factors which favor or dis-favor this possibility. The potential problems of either public ownership, and public utility operation of the milk business are cited. The inherent differences that exist between the normal utilities, as gas, electric and water companies and a unified milk business, and the relative effect of factors on the operation of these businesses are presented. Lastly, who stands to benefit by the institution of a unified milk distribution system? Certain groups, as for example organized producers, it is stated, are better off with the competitive rather than with the unified system.

Technically, there is little doubt that unification of operations could bring about reduction of costs from  $1\frac{1}{2}$  to  $2\frac{1}{4}$  cents per quart. But the social implications, the selection of capable and honest management, freedom from political interference, price determination and the like are activities which are unpredictable, and which may defeat any technical advantages of unification. The outlook for a unified system is concluded. This book is readable, understandable, impartial in presentation. It is of value to all associated with the dairy industry, and those in or aspiring to public office, as an excellent review and discussion of the contemporary social problem of the industry.

K.G.W.

#### 487. Fruit Pectins. Their Chemical Behavior and Jelling Properties.

C. L. HINTON, Dept. of Scientific and Industrial Research, Great Britain. Food Investigation Special Report No. 48. 96 pages, Price \$1.75. Published by Chemical Publishing Co., Inc., 148 Lafayette St., New York.

The information presented is based on work conducted by the staff of the British Association of Research for the Cocoa, Chocolate, Sugar Confectionery and Jam Trades. The divisions in the book include the constitution, characterization, measurement of jelly-forming capacity and chemical properties of pectins. The data on pectins from five fruits (oranges, lemons, apples, gooseberries and strawberries) is presented. The effects of heating, acids, alkalies, and salts, the methods of extraction and the action of pectase on the various pectins is reviewed. The data is excellently illustrated by the use of graphs (13) and tables (42). The jelling power of pectins is dependent upon its inherent quality (some are naturally weak, others naturally strong), the degree of heating to which it has been subjected, and effects of treatment with strong acids at ordinary temperatures or less. The jelling power of pectins as exhibited by the pectins in jellies is further affected by the degree of de-esterification caused by acids or pectase action,

pH conditions as fixed by buffering constituents or neutralization of the free acid of the pectins, and the beneficial effect of some salts (calcium chloride, for example, increases the pH range over which a pectin makes suitable jellies).

There is no specific reference to the use of pectins in milk products. Nevertheless, the properties of the pectins are very well covered and the information will be of use to manufacturers of, and research workers in, products such as fruit ice cream pies, fruit ice cream flavors, and special products as spreads.

K.G.W.

## BACTERIOLOGY

**488. Oxidase-Positive Bacteria in Dairy Products.** C. H. CASTELL. Can. Dairy and Ice Cream J., 19: 5, 26. 1940.

"Oxidase-producing" bacteria are responsible for the spoilage of fatty foods by oxidation. They also hydrolyze the fat and bring about rapid decomposition of proteins. Those giving very strong reactions included all the members of the *Pseudomonas* and *Achromobacter* genera. Individual members showed the following defects in cream and butter: potato odor, surface taint, cheesiness, limburger, putridness and rancidity. Organisms giving a less marked reaction were those of the *Brucellus* and *Alcaligenes* genera and the Gram negative organisms from soil and diseased plants. Oxidase-negative organisms included most coccus types and a large majority of spore-forming bacteria. Most molds isolated from butter were strongly oxidase-positive. No oxidase-positive organisms were found in aseptically drawn milk. Oxidase-positive organisms were found in great numbers in molasses, corn silage, dust, decaying plant tissue, soil and surface waters and butter. Directions for making the oxidase test are given.

O.F.G.

**489. The Effect of pH on Growth and Gas Production by Streptococci and Lactobacilli.** J. G. DAVIS AND C. C. THIEL, The Nat'l Institute for Research in Dairying, Univ. of Reading, Reading, England. J. Dairy Res., 10: 455-463. 1939.

The pH ranges of growth of streptococci and lactobacilli in dextrose yeast casein digest broth was studied. Nearly all the group I and II streptococci grew at pH 8.8, but only the enterococci grew at pH 4.8. All group III types (heterofermentative) grew at pH 4.4. Only a few lactobacilli grew at pH 8.8 but most were able to grow at pH 4.0. *S. cremoris* could be differentiated from *S. lactis* by failure to grow at pH 9.2.

S.T.C.

**490. Bacteriophage-organism Relationships in the Group of Lactic Streptococci.** H. R. WHITEHEAD AND G. J. E. HUNTER, Dairy Research Institute, Palmerston North, New Zealand. J. Dairy Res., 10: 403-409. 1939.

Methods are described for the isolation and purification of bacteriophages

active against lactic streptococci. The relationships between nine apparently distinct phage races and eleven strains of lactic streptococci were studied. The phages were found to show a tendency toward strain specificity, although some of the races attacked up to four different strains. Resistant strains usually were found to arise on continued incubation of lysed cultures. Cross resistant tests, in those cases where two or more phages act on one strain of streptococci indicated that the relationships between the phages does not follow any simple rule.

S.T.C.

**491. Biennial Reviews of the Progress of Dairy Science. Section B. Bacteriology and Mycology Applied to Dairying.** J. Dairy Res., 10: 515-549. 1939.

A review of literature published principally during 1937-1938 under the following headings:

- I. Milk control
  - Technique
- II. Micro-organisms in milk and milk products
  - (a) *Coli-aerogenes* group
  - (b) Spore-bearing bacteria
- III. Lactic acid and allied fermentations
  - (a) Bacterial metabolism
  - (b) Lactic acid bacteria
  - (c) Starters
  - (d) Cheese
  - (e) Butter
- IV. Pasteurization and other processes
  - (a) Pasteurization
  - (b) Other processes
  - (c) Canned and dried milk products
  - (d) Ice cream

S.T.C.

**492. Examples of Variation within Pure Cultures of *Streptococcus cremoris*.** G. G. E. HUNTER, Dairy Research Institute, Palmerston North, New Zealand. J. Dairy Res., 10: 464-470. 1939.

Variability within pure strains of *Streptococcus cremoris* was demonstrated. In one strain changes in acid producing power were made evident by changes in colony form. One variant failed to ferment lactose but was still susceptible to the specific race of bacteriophage and exhibited the same morphology as the parent culture. Variation was well marked within some strains, especially in regard to acid production, response to high temperature conditions, power to produce ropiness in milk and degree of resistance to phage attack.

S.T.C.

493. **The Enrichment of aerogenes-cloacae Types in Milk Held at Low Temperatures: with Observations on the Relative Rates of Growth of aerogenes-cloacae and B. coli Types in Milk at Different Temperatures.** JAMES F. MALCOLM, Dept. of Bacteriology, The West of Scotland Agricultural College, Glasgow, Scotland. J. Dairy Res., 10: 410-425. 1939.

Cultures of *B. aerogenes*, *B. oxytoccus* and *B. cloacae* types were found as a rule to multiply much more rapidly when grown at 17° C. in sterilized, partly sterilized or raw milk than those of the *B. coli* types. Counts were made by the plate method using milk agar. With mixed cultures grown in milk the *aerogenes-cloacae* type multiplied more rapidly at 17 and 22° C. than the *B. coli*, while at 30 and 37° C. the reverse was the case. Twelve specimens of bovine feces were inoculated into raw milk and the cultures kept at 17° C. for 36 hours. *Aerogenes-cloacae* types occurring in the feces became enriched in the milk, the coliform flora at the end of the incubation period frequently consisting chiefly of these types.

The author explains the greater incidence of *aerogenes-cloacae* types in summer milk in Scotland as compared with winter milk, as due to enrichment of these types at the temperatures of holding commonly employed in the summer. Such enrichment does not occur in the winter since the holding temperature as a rule is so low that there is little or no proliferation of any coliform types.

S.T.C.

#### BUTTER

494. **A Discussion of Some Phases of Butter Deterioration.** E. G. HOOD, Elgin Annex, Ottawa, Ont. Can. Dairy and Ice Cream J., 19: 4. 54. 1940.

Real quality in butter means quality at the time the butter is consumed. Without quality cream there can be no quality butter. Flavors that appear in the cream are nearly certain to reappear in the butter unless specially treated and this treatment adds to the cost of manufacture. The age of the cream is one of the major factors controlling the quality of butter since time is required for harmful bacterial action and oxidation of the fat to take place. Not all sweet cream makes good flavored butter. Chemical deterioration resulting in off-flavor is due to oxidation, hydrolysis or catalysis of any of the constituents normally present in milk. Considerable quantities of butter now find their way into the second grade class as the result of surface flavor defects. Bacterial deterioration of the fat and protein of butter results in further flavor defects.

O.F.G.

495. **The Neutralization of Cream for Buttermaking. I. The Accuracy of Acid Reduction by Various Neutralizers.** R. C. TOWNLEY AND I. A. GOULD. Can. Dairy and Ice Cream J., 19: 5, 54. 1940.

The accuracy of acid reduction is dependent upon (a) the type of neu-

tralizer used, (b) the degree or extent of acid reduction, and (c) the temperature of pasteurization. Each of the neutralizers showed a distinct variation from the linear reduction of acidity. As the acidity ranges were lowered all of the neutralizers decreased in efficiency. Caustic soda and the limes were more efficient than the carbonates in reducing acidity throughout the entire range. The pH of the butter was always above that of the cream from which it was made.

O.F.G.

- 496. "Vacreating" Cream.** R. W. BROWN, Univ. of Manitoba, Winnipeg, Man. *Can. Dairy and Ice Cream J.*, 19: 3, 21. 1940.

Cream for buttermaking was treated in a "vacreator" which is essentially a flash pasteurizer working under reduced pressure. It is claimed that the cream reaches the pasteurizing temperature (201° F.) in one second and leaves the pasteurizing chamber in two seconds after entering. The cream passes from the pasteurizer to a second chamber under a vacuum of about 20 inches where volatile substances are drawn off. Butter made from "vacreated" cream of clean flavor and acidity was 1.08 points better in flavor score at the end of 4-6 weeks held at 45°-50° F. than was butter made from the same cream but pasteurized in the usual way. Butter made from high-acid stale cream and held under the above conditions showed an advantage of 2.08 points in flavor score in favor of the vacreated cream. The advantage for vacreated butter made from feed and weed flavored cream was 3.59 point, and from metallic flavored cream, 2.46 points. More efficient neutralization was brought about in vacreated cream than in pasteurized cream. O.F.G.

- 497. Preparing Prize-winning Butter.** L. C. THOMSEN, Univ. of Wis., Madison. *Nat. Butter and Cheese J.*, 31: 7, 10. July, 1940.

Suggestions are given to the man who is making butter for a contest. They cover the selection, standardization, pasteurization, cooling, ripening, and churning of cream; washing, salting and working the butter; finishing, filling, identification and shipment of the tub.

W.V.P.

## CHEESE

- 498. Factors Influencing Cheesemaking Methods and Yields.** W. S. ARBUCKLE. *Can. Dairy and Ice Cream J.*, 19: 4, 60. 1940.

The author describes some of the physico-chemical phenomena and relationships in milk and in cheese. The roles of fat, casein, ash and lactose and their influence on color, flavor and body of cheese are discussed. Water has a definite influence on the body of cheese because it supplies a medium for the chemical breakdown of the casein during ripening. The problems of pasteurization of milk for cheesemaking, of mastitic milk, of the treatment of the cheese curd and of the influence of temperature on ripening are discussed.

O.F.G.

499. **The Bacterial Flora of New Zealand Cheddar Cheese.** I.R. SHERWOOD, Dairy Research Institute, Palmerston North, New Zealand. J. Dairy Res., 10: 426-448. 1939.

Seven hundred and twenty strains of lactic acid bacteria isolated from thirty-six typical cheddar cheeses were classified. *Streptobacterium plantarum* was found to be the dominant organism in New Zealand cheese. *Streptobacterium casei* occurs much less frequently, while betabacteria and betacocci are found in still smaller numbers. The flora of good quality cheese was found to consist mainly of one or two varieties of *Streptobacterium plantarum*, often associated with *Streptobacterium casei* or a small proportion of betabacteria.

*Streptobacterium casei* in general was found to be beneficial to cheese quality. The strains of *Streptobacterium plantarum* isolated were grouped in four varieties, mainly on the basis of sugar reactions. One variety was beneficial to cheese quality, another had very little effect, while the remaining two varieties produced serious defects—bad flavors, discoloration and, occasionally, open texture. The same defects were produced by the most of the strains of betabacteria and betacocci when present in large numbers.

S.T.C.

500. **Lactic Acid Bacteria in Relation to Cheese Flavor. II. Observations on the Inoculation of the Milk Employed in Cheese Manufacture with Lactobacilli.** I. R. SHERWOOD, Dairy Research Institute, Palmerston North, New Zealand. J. Dairy Res., 10: 449-454. 1939.

Attempts were made to improve the flavor of cheese by inoculation of good quality pasteurized cheese milk with selected strains of *Streptobacterium casei* and *Streptobacterium plantarum*. Large inoculae imparted a sharpness to the flavor of the cheese especially during the early stages of ripening. The best results were obtained when the inoculum was such that the numbers of lactobacilli added were not much greater than the numbers of such organisms "naturally" present in raw milk, e.g., 10 ml. of clotted culture to 80 gallons of cheese milk. The author suggests that under factory conditions the lactobacilli might be propagated by the incorporation in the "mother" starter (enriched with a vegetable extract) of suitable strains of *Streptobacterium plantarum*.

S.T.C.

501. **Mastitis and Cheese Milk.** C. K. JOHNS, T. J. HICKS, AND C. A. GIBSON, Central Experimental Farm, Ottawa, Ont. Can. Dairy and Ice Cream J., 19: 5, 19. 1940.

Yields of cheese from "abnormal" and "agalacteive" milk were found to be lower than those from normal milk by about 5 per cent. No appreciable differences in the quality of the cheese from the 3 groups was noted. All

milks appeared perfectly normal and abnormalities were detected by biochemical or bacteriological tests. Udder injections increase the catalase content of milk and a method for measuring this enzyme seemed most promising for factory use.

O.F.G.

- 502. Manufacture and Packaging of Cheese in a Labeled Consumer-size Package.** H. L. WILSON, Bureau of Dairy Industry, U. S. Dept. of Agr. Milk Dealer, 29: 10, 94-101. July, 1940.

A description is given of the proper methods to use in the processing, canning, and curing of cheese in order to insure a product of good uniform quality. The author concludes with the following statements: "High-acid cheese or cheese made from low-grade milk should not be canned. High-acid cheese never develops the characteristic flavor or improves with aging. The quality of the cheese varies directly with the quality of the milk from which it is made."

C.J.B.

- 503. The Application of the Frozen Pack Method to Preservation of Soft Cheese.** N. S. GOLDING AND MAX E. MORGAN, Dept. of Dairy Husbandry, Agr. Exp. Sta., State College of Washington, Pullman, Wash. Milk Dealer, 29: 9, 42-46. June, 1940.

A report of experimental work to determine the application of the frozen pack method to the preservation of soft cheese. The authors conclude that Gervais cream cheese, Neufchatel and Neufchatel with pimento flavor are suited for frozen pack preservation. The limit of time for such storage under these conditions has not been determined, but a period of 10 weeks can be considered safe. Off-flavors and defective cheese, in general, will retain these defects during storage.

C.J.B.

- 504. Fly Control in Cheese Factories.** HUGH GLASGOW, N. Y. State Agr. Exp. Sta., Geneva. Nat. Butter and Cheese J., 31: 7, 23. July, 1940.

Guarding the cheese factory from flies is made difficult by the number of species involved and their varied breeding habits. The house fly, blow flies, blue bottle flies and little house fly originate in filth or decaying animal matter. Immature midges live in ponds or streams while fruit flies or vinegar gnats develop in fermenting materials like fruits or milk refuse. The cheese maker should keep the flies out of the factory by keeping it dark by drawn shades and use of orange colored lights; by excluding them with screens supplemented by electrical screens; by eliminating breeding places; by using strong fly sprays and repellants around the outside whey tank and receiving platform; and by using odorless fly sprays and fly paper within the plant.

W.V.P.

## CHEMISTRY

- 505. Analysis of Commercial Fats and Oils.** Report of Amer. Chem. Soc. Committee. Ind. and Eng. Chem., Anal. Ed., 12: 7, 379-384. 1940.

This report gives details of methods of analysis which have been investigated and studied by the committee for a period of 2 to 3 years. The six methods which are recommended for adoption are: (1) the titer which gives the solidification point of fatty acids and in which a few changes have been made; (2) the modified Gardner break test which is applicable to crude soybean oil; (3) the detection of tristearin in lard which is applicable to the detection of foreign fats containing tristearin (beef fat) in unhydrogenated pork fats; (4) the Villavecchia test for the qualitative detection of sesame oil; (5) the method for calculation of the hydroxyl value for fat or wax which has been included with the acetyl value determination; (6) the smoke, flash and fire points applicable to animal and vegetable oils and fats.

B.H.W.

- 506. Assay of Vitamin A with the Photoelectric Colorimeter.** R. B. FRENCH, Fla. Agr. Exp. Sta., Gainesville, Fla. Ind. Eng. Chem., Anal. Ed., 12: 6, 351-352. 1940.

The accuracy of measuring vitamin A using the Cenco photelometer and the antimony trichloride reaction was investigated. The photelometer gave consistent, reproducible results in spite of the fact that the characteristic blue color faded rapidly. With close timing replicate determinations checked well.

B.H.W.

- 507. Making Casein Fiber.** E. O. WHITTIER AND S. P. GOULD, Bureau of Dairy Industry, U. S. D. A., Washington, D. C. Ind. Eng. Chem., 32: 7, 906-907, 1940.

To make casein fiber casein is precipitated at a greater acidity than is ordinarily used in the manufacture of casein for other purposes. The kind and amount of acid used affects the fiber made from it. The casein is dissolved in an alkaline solvent such as NaOH, Na<sub>3</sub>PO<sub>4</sub>, NH<sub>4</sub>OH. Salts of metals such as aluminum, calcium and barium should be present in the casein solution to increase the strength of the fiber. Fat acids such as oleic acid, linseed oil acids or others are added to the casein solution to increase flexibility. The casein solution is extruded through fine openings into a precipitating bath containing such an acid precipitant as sulfuric, phosphoric or acetic acid. The presence of 20 per cent glucose in the bath increases the speed of dehydration of the fiber. Formaldehyde or other aldehydes further increase the strength of the fiber and oil emulsions increase its softness and flexibility. Two examples of recipes giving relatively strong flexible fiber are given.

B.H.W.

- 508. Lactic Esters, Preparation and Properties.** LEE T. SMITH AND H. V. CLABORN, Bureau of Dairy Industry, U. S. D. A., Washington, D. C. Ind. Eng. Chem., 32: 5, 692-694. 1940.

Since lactic acid may be manufactured from whey its utilization is important to dairy processors.

This paper reports the general methods of preparation and the properties of the 3 types of lactic esters which may be made from lactic acid. Some of the methods of preparation have commercial possibilities. A table giving some physical properties of 30 esters is given. B.H.W.

### CONCENTRATED AND DRY MILK; BY-PRODUCTS

- 509. Une Méthode Rapide pour l'Estimation des Propriétés Élastique et Plastique de la Caséine à la Pression.** (A Rapid Method of Estimation of the Elastic and Plastic Qualities of Rennet Casein.) G. GENIN. Le Lait, 20: 291-296. May-June, 1940.

The use of casein in the plastic industries is hindered because of the marked variation which exists from one batch of raw casein to the next. C. A. Cooper (Brit. Plastics, March, 1939, p. 572) found that the Scott apparatus used by rubber manufacturers could be adapted to determine the deformations and elasticity of casein samples under heat and pressure.

Cylinders of casein are moulded under standard conditions and then subjected to three minutes pressure at 120° C. The temporary deformation, permanent deformation and elasticity can then be obtained by measuring the changes in height which occur in the test pieces.

Highly elastic caseins are difficult to mill and shape. There is no strict relationship between the elastic properties of casein and pH although most of the superior New Zealand and Australian samples gave higher values for both pH and ash content. O.R.I.

- 510. Le Contrôle Hygiénique du 'Lait Concentré Sucré.'** (The Hygienic Control of Sweetened Condensed Milk.) C. A. CECILIA, Vet. School, Madrid. Le Lait, 20: 271-279. May-June, 1940.

During the Spanish civil war, large quantities of sweetened condensed milk of foreign origin were used in Spain. Defects present in 149 samples studied by the author are reported upon. Cans were examined for swelling and leaking externally, and for odor, color, taste and abnormal viscosity after incubation. Fifty ml. of a 1:5 dilution was also examined by inoculation in peptone water, Breed count, plate count on glucose, agar, reductase, acidity and sediment.

In many cases, swollen cans contained rancid, fruity, cheesy or sour milk. Bacterial flora included cocci, Torula and spherical yeasts, spore-forming rods, and some moulds. Counts were much higher than are usually

reported for sweetened condensed milk. Excessive thickening usually accompanied high counts. Oily and tallowy flavors were only reported in five samples.

Incubation of suspicious cans for 48 hours and determination of acidity appeared to be the most reliable method of testing the quality of this product.

O.R.I.

**511. The Denaturation of the Soluble Proteins of Whey by Heat.** O. R.

IRVINE AND W. H. SPROULE, Ontario Agr. College, Guelph, Ont.  
Can. Dairy and Ice Cream J., 19: 3, 62. 1940.

Pasteurization temperatures higher than 155° F. result in the denaturation of whey albumin. It was found that long holding times did not greatly increase the percentage of protein denatured except when the pasteurization temperature was 74.9° C. and the pH 6.35. An appreciable amount of protein denaturation may be expected even at a relatively low temperature of pasteurization. Denaturation can be greatly reduced if pasteurization of the whey can be carried out at a lower level of acidity.

O.F.G.

**512. Factors Affecting the Solubility of Milk Powders. IV. The Influence of Speed and Duration of Stirring on Solubility with Description of a Rapid Method for Solubility Determinations.**

J. R. HOWAT, J. A. B. SMITH, R. WAITE, AND N. C. WRIGHT, The Hannah Dairy Research Institute, Kirkhill, Ayr. J. Dairy Res., 10: 498-514. 1939.

Increases in the speed and duration of stirring were found to increase the apparent solubility of milk powders. It was concluded, however, that 30 seconds shaking of the powder reconstituted in a 10 per cent mixture was sufficient to dissolve the truly soluble portion of the dried milk, but that the protein which has become denatured during the drying process tends to pass into solution with increased speed and duration of stirring.

The following method is suggested for solubility determinations: "1 g. of the powder to be tested is weighed into a 15 ml. centrifuge tube. About 2 ml. of water are added from a burette and the mixture stirred well with a glass rod which had been previously wetted. When all the powder has become thoroughly moistened, more water is added until a total of 9 ml. has been run in, the stirring rod being washed with the last few ml. of water. The tube is then stoppered and kept in a water bath either at 20 or 50° C. for 5 min. and is then shaken rapidly for 1 min. The shaking speed will affect to some extent the solubility figure finally obtained, but if the process be carried out as vigorously as possible, involving some 5-6 complete double excursions per second, very close agreement is obtained by different workers for the same sample. If it is desired to estimate the solubility at 50° C. the tube is shaken inside a container lined with cotton

wool to conserve the heat. The tube is then cooled to room temperature and centrifuged, the supernatant layer poured off as completely as possible (including the fat if the sample is a whole-milk powder), and its total solids content estimated by the rapid method of Golding. The ratio of the dissolved solids to the solids initially present (expressed as a percentage) is taken as an index of the solubility. The initial solids must be corrected for moisture content."

At higher solubilities the sediment volume found in the sediment method was shown to be a fairly reliable guide to small changes in solubility, but was much less accurate in the lower solubility ranges. S.T.C.

NOTE: This method is in variance with the accepted method recommended by the Dry Milk Institute.

## DISEASE

- 513. Action of Gramicidin on Streptococci of Bovine Mastitis.** R. B. LITTLE, R. J. DUBOIS, AND R. D. HOTCHKISS, Depts. of Animal and Plant Pathology and the Hospital, Rockefeller Inst. for Med. Res. Proc. Soc. Exp. Biol. and Med., 44: 444. 1940.

An attempt was made to determine if gramicidin, an alcohol-soluble substance isolated from cultures of a sporulating bacillus, would destroy the streptococci causing mastitis when injected into the infected quarter. Repeated treatments of 2 animals suffering from chronic mastitis failed to eliminate permanently the streptococci from the infected quarters. Three cows with 9 infected quarters were treated with gramicidin. In 2 quarters repeated treatment failed to eliminate the streptococci while 5 treatments were required to sterilize one of the quarters. Repeated treatments resulted in a decreased milk flow. In the remaining 6 quarters (in 5 cases after a single injection) the streptococci disappeared without an appreciable decrease in milk secretion. It was concluded that "Before the effectiveness of gramicidin in the control of bovine mastitis can be determined, a larger number of animals must be treated and observed over a longer period of time."

R.P.R.

## FEEDS AND FEEDING

- 514. Chemical Changes in Phosphoric Acid Silage.** EDOUARD PAGÉ AND L. A. MAYNARD, Cornell Univ., Ithaca, N. Y. Ind. Eng. Chem., 32: 8, 1140-1143. 1940.

Grass silage, constituting of 62.2 per cent clovers, 19.8 per cent alfalfa, 16.2 per cent grasses and 1.8 per cent weeds and from 0 to 24 lbs. of 68 per cent phosphoric acid per ton of silage added to different layers, was subjected to chemical analysis after 8 to 9 months in the silo. The layers of silage were separated by waterproof rubber sheets and it was found that

the position of the layer markedly influenced the quality of the final product. To counteract this effect of position, for experimental purposes, the treatments applied to the lower layers were repeated in the upper half of the silo. The appearance and odor of all layers were good but analysis showed that the higher quality of some samples was due to the presence of lactic as well as phosphoric acid. The chemical evidence shows phosphoric acid to be of definite value as a preservative but that its action must be supplemented by a strong lactic acid production for best results. . . . B.H.W.

- 515. The Effect of Increased Iodine Feeding upon the Iodine Content of Cow's Milk.** N. L. MATTHEWS, G. M. CURTIS, AND J. H. MEYER, Dept. of Research Surgery, The Ohio State Univ., Columbus, Ohio. J. Dairy Res., 10: 395-402. 1939.

The milk and blood iodine of a herd of thirty Guernsey and thirty Holstein cows was determined previous to and during prolonged increased iodine feeding to one-half of the herd. The increased amount of iodine was mixed with the grain ration, 3.2 mg. per cent of iodine as potassium iodide being added.

The blood iodine of the iodized cows was greatly and uniformly increased. The milk from the iodized cows contained 7 to 26 times as much iodine as that from the control cows. An average milk iodine of 80 mg. per cent was obtained from the iodized cows throughout a mid-year period of 5 months. During late spring, however, the milk iodine from both the iodized and control cows was unusually low. During early autumn the milk iodine from the iodized cows was low. S.T.C.

- 516. Grass Silage.** G. BOHSTEDT, W. H. PETERSON, AND F. W. DAFFEE, Univ. of Wis., Madison, Wis. Circ. 299, 20 pages. May, 1940.

A popular treatise dealing with the characteristics of, methods of harvesting, preserving, cost of putting up and the feeding of grass silage.

W.E.P.

- 517. Home-grown Grains and By-Products as Feeds for Dairy Cattle.** D. L. FOURT AND F. W. ATKESON, Univ. of Idaho. Ext. Circ. 68. Revised April, 1940.

In addition to a general consideration of the characteristics of a good ration suitable grain mixtures are given for roughages of different protein contents.

W.E.P.

- 518. Dehydrated and Sun-Cured Hay.** S. I. BECHDEL, A. W. CLYDE, C. O. CROMER, AND P. S. WILLIAMS. Pa. Agr. Exp. Sta. Bull. 396. June, 1940.

Tests with two types of artificial driers are reported: the rotary drum,

high temperature style; and the conveyor, low temperature style. Hay of superior feeding value for dairy animals can be produced regardless of the weather. The cost of drying is as yet too high to compete with sun-cured hay for dairy stock, though it is being used considerably in poultry feeds. The crushing process for hastening natural drying has much promise of usefulness in the humid sections of the country since it reduces damage to hay by dews and rains. Under favorable conditions, hay cut with a crusher-mower in the morning can be stored that afternoon, whereas mower-cut hay must be left in the field until the next day. Author's Abstract

- 519. Bone Meal versus No Bone Meal in the Dairy Ration.** S. I. BECHDEL, P. S. WILLIAMS, J. F. SHIGLEY, AND A. A. BORLAND. Pa. Agr. Exp. Sta. Bull. 395. June, 1940.

A total of 117 lactation periods of 33 dairy cows was studied in a comparison of bone meal versus no bone meal in the concentrates fed. The data obtained on the weight and health of calves and the number of services necessary for conception are slightly favorable to bone meal. The results on milk production lead to the conclusion that the ordinary Pennsylvania dairyman, unless he has unusually high producers, does not need to add bone meal to the dairy ration. Author's Abstract

### FOOD VALUE OF DAIRY PRODUCTS

- 520. Le Taux de la Vitamine C du Lait de Vache et son Importance dans l'Alimentation Infantile.** (The Vitamin C Content of Cow's Milk and Its Importance in Infant Feeding.) M. GUIGOZ. Le Lait, 20: 279-286. May-June, 1940.

Certain clinicians claim that children can be protected from scurvy with an intake of as little as 5 mgm. of ascorbic acid per day in contrast to a theoretical requirement of 20-25 mgm. suggested by other authorities. Since mother's milk is almost twice as rich in this vitamin as is the average cow's milk, it is suggested that the period of breast feeding builds up a reserve in the infant sufficient to protect it through the first few months of life. In such cases, cow's milk contains sufficient ascorbic acid to assure protection of the healthy infant against scurvy throughout the time that it is exclusively milk fed. A good bibliography of recent literature is included in this report. O.R.I.

- 521. The Effect of Light on the Vitamin C of Milk in Different Containers.** J. HOUSTON, S. K. KON, AND S. Y. THOMPSON, National Institute for Research in Dairying, Univ. of Reading, Reading, England. J. Dairy Res., 10: 471-474. 1939.

Twice as much vitamin C survived in milk in wax impregnated cartons

exposed to the action of light as in clear glass bottles. Brown glass bottles showed little destruction, green glass bottles considerably more, although less than wax impregnated cartons.

S.T.C.

**522. Effect of Irradiated Milk on Storage of Nitrogen and Acid Base Minerals in Children.** FRANCES C. HUMMEL, HELEN A. HUNSCHER, AND ICIE G. MACY, Children's Fund of Michigan, Detroit, Mich. Am. J. Diseases of Children, 58: 753. 1939.

Observations were made on the mineral storage of eight children 5 to 8 years of age during test periods of 20 to 60 days, each child receiving first non-irradiated milk and later irradiated milk of the same kind and quantity. Three of the children were given fluid milk, 3 were given evaporated milk, and 2 were given a combination of both kinds.

From the standpoint of the average actual daily retention alone, it did not appear that the additional vitamin D in irradiated milk (90 to 96 and 180 to 190 U.S.P. units daily) had any nutritive advantage in the deposition of minerals. On the other hand, the children showed a more rapid rate of growth in length during the periods when vitamin D was the only factor added. The increase in rate of skeletal growth was, in general, accompanied by a higher calcium-phosphorus ratio in the retention. This indicates a stimulus to formation of bone and by the decrease in retention of nitrogen, phosphorus, and sulfur a shift from the formation of soft tissue to that of skeletal structure. From these cumulative results it seems justifiable to conclude that vitamin D supplements in the form of irradiated milk included in the dietary known to be nutritionally good and given to children who have good health not only exert a regulating influence on the growth impulses but stimulate skeletal development.

W.H.R.

**523. Influence of Fluid and Evaporated Milk on Mineral and Nitrogen Metabolism of Growing Children.** HELEN J. SOUDERS, HELEN A. HUNSCHER, FRANCES C. HUMMEL, AND ICIE G. MACY, Children's Fund of Michigan, Detroit, Mich. Am. J. Diseases of Children, 58: 529. 1939.

The nitrogen and acid-base mineral balance study was conducted on 3 healthy children 4½ to 6 years of age, receiving a basal diet of adequate requirements. During the first period of 25 to 40 consecutive days of metabolic study, 400 grams of plain fluid milk were ingested daily by each child. During the 20 to 25 days immediately following, a similar amount of dilute (1:1) evaporated milk was substituted and for the next 25 days irradiated evaporated milk was fed.

An added impetus to the formation of soft tissue resulted from the substitution of evaporated for fluid milk as shown by the parallel increases in retention of nitrogen, sulfur, and potassium.

When irradiated evaporated milk was included in the diet the increased calcium-phosphorus ratios of the retention, the higher levels and more consistent trends of the acid base balances, and the increase in rate of gain of recumbent length all indicated a more rapid and stable rate of formation of bone.

W.H.R.

### ICE CREAM

524. **Using Frozen Fruit Pulps in Ice Cream Making.** D. C. SORBER. Can. Dairy and Ice Cream J., 19: 5, 22. 1940.

It is now possible by means of an inexpensive freezing process to preserve the important characteristics of fresh fruit for use in making ice cream at seasons of the year when the fresh fruit is not available. A long list of satisfactory varieties of fruit is given. Nothing but fully ripened soft fruit with high flavor should be used. Directions are given for the preparation of the fruit and the proportion of sugar to use. Contamination of the mix or of the fruit with copper should be avoided since several kinds of fruit intensify the reaction of copper in the development of oxidized flavor. Rapid handling is recommended to insure the preservation of the maximum quality that exists in fresh fruit.

O.F.G.

525. **Some Causes of Shrinkage in Package Ice Cream.** ROLAND KOHLER, 2101 S. Los Angeles St., Los Angeles, Calif. Can. Dairy and Ice Cream J., 19: 3, 26. 1940.

Factors which cause the shrinkage of ice cream are, (1) mix ingredients and mix composition, (2) processing mix, (3) freezing process, (4) hardening process, (5) transportation or delivery, and (6) storage and cabinets. Mix composition is the least expected and the hardest to control but it was found that the stability of milk solids-not-fat, especially those from condensed skim milk, play an important role in shrinkage. Tests indicated that adjustments of the stability of the proteins could be made through the addition of calcium, sodium, phosphates and citrates. Factors which tend to increase instability, and thus shrinkage, are high temperature pasteurization, homogenization at high pressures, the freezing process, especially high speed continuous freezing, and hardening at especially low temperatures.

O.F.G.

526. **A Discussion of Sweetening Agents for Ice Cream.** P. H. TRACY, Univ. of Illinois, Urbana, Ill. Can. Dairy and Ice Cream J., 19: 3, 58. 1940.

Sugar adds to the food value, improves palatability and lowers the freezing point of ice cream. The sweetening value of cane and beet sugar is the same. Dextrose, made from corn, has come to replace sucrose to some

extent in ice cream manufacture. It is more easily assimilated by the body but in water solution it has a sweetening value of about 70 as compared to 100 for sucrose. Its sweetening value is increased when used in conjunction with sucrose in ice cream. Its use will not seriously affect the time required to freeze and whip the mix but the drawing temperature will be about one degree lower. When used to replace about 25 per cent of the sucrose, it will have no detrimental effect on the flavor or body of ice cream. A new type of corn syrup, "Sweetose," has been developed recently that may have considerable use in the manufacture of ice cream. The body of ice cream containing "Sweetose" is noticeably smoother than the body of ice cream sweetened entirely with sucrose. The author recommends about 9 per cent honey with 8 per cent sucrose and no additional flavor to get the best honey flavor in ice cream.

O.F.G.

## MILK

**527. Milk and the Public Health.** R. O. DAVIDSON. Can. Dairy and Ice Cream J., 19: 4, 68. 1940.

The importance of safe milk for public consumption is emphasized. "Clean milk does not necessarily indicate a 'safe milk,' consequently something else, other than sanitary conditions, is required; that something is heat treatment of the milk to kill pathogenic organisms." The author feels that nutritive losses in the pasteurization of milk are not important. O.F.G

**528. Safeguarding the Milk Supply of Large Cities.** J. C. GEIGER AND B. G. ENGLE. Can. Dairy and Ice Cream J., 19: 5, 30. 1940.

This is a summary of the needs for the methods of safeguarding the milk supply of large cities. The vital steps in offering the public a safe milk are:

- (1) All milk and milk food products should be produced by healthy herds free from tuberculosis.
- (2) All milk and milk food products should be produced from "Grade A" farms under the inspection service of the local department of health.
- (3) All milk should be pasteurized with a rigid system of inspection of the plants.

O.F.G.

**529. Factors Affecting the Whipping Quality of Whipping Cream.** C. J. BABCOCK, Bureau of Dairy Industry, Washington, D. C. Can. Dairy and Ice Cream J., 19: 5, 40. 1940.

Raw cream is slightly superior for whipping purposes but the safeguards of pasteurization should be considered. Homogenization destroys the ability of cream to incorporate air and thus impairs its whipping quality. Aging will improve whipping quality but a period longer than 24 hours is not

recommended. Increasing the acidity does improve whipping quality. To get good whipping the temperature should not be above 50° F. O.F.G.

- 530. Titration des *B. Cole Aerogenes* dans les Laits.** (Detection of *Coli-aerogenes* in Milks.) E. PIRAX, Exp. Sta., Gembloux, Belgium. *Le Lait*, 20: 257-271. May-June, 1940.

The presence of organisms of the *Escherichia-Aerobacter* group has significance in pasteurized milk, in butter, and in milk for cheese manufacture. Unless thermoduric strains are present, organisms of this type should not survive pasteurization. Positive differentiation between *Escherichia* and *Aerobacter* groups is difficult due to the many atypical strains found in nature.

A presumptive test for the presence of colon organisms must show the production of acid and gas in a liquid medium. Lactic acid production by lactics must be inhibited if growth of the colon types is to take place. Crystal violet, sodium formate, bile salts, etc., have been suggested as suitable antiseptics for this purpose.

In the study, tryptoflavine was found to be a satisfactory lactic inhibitor when used in a concentration of 1 or 2 parts per 100,000 parts of milk. One part of a 1 per cent neutral red solution in 100 parts of milk, was used as a reduction indicator. The milk was enriched by the addition of yeast extract at the rate of 2 per cent. In order to more accurately detect gas production the medium in the tubes was covered with a layer of melted paraffin. Incubation was at 37° C. for 24 to 48 hours.

If it is necessary to determine the presence of these organisms in low dilution, the sample can be diluted in physiological saline and the above medium prepared using sterile milk.

Confirmatory tests recommended include: Gram staining, or plating on Difco Violet Red Bile Agar, on Endo's medium or on Levine's medium.

O.R.I.

- 531. La Réglementation du Lait Malpropre.** (The Control of Unclean Milk.) L. HOTON. *Le Lait*, 20: 287-291. May-June, 1940.

The advisability of attempting to legally define "clean" milk is discussed. Such a definition is very difficult and many primitive and unsatisfactory regulations are cited which have been retained in the statutes. Some of the more obsolete statutes prohibit filtering. Several suggestions are put forward for revising these laws.

O.R.I.

- 532. Milk and Child Welfare.** A. B. SCHWARTZ, Chairman, Child Welfare Committee, State Medical Society of Wisconsin. *Milk Dealer*, 29: 9, 110-113. June, 1940.

A brief history is given of the role which milk has played in child welfare.

C.J.B.

- 533. What the Consumer Believes.** EDWARD FISHER BROWN, Milk Research Council, New York City. *Milk Dealer*, 29: 9, 114-117. June, 1940.

A psychological study of the attitudes of various lower-income group New York City housewives showed that: Most New York City housewives were favorably disposed or neutral toward their milk companies. More than two-thirds of the group, 719 out of 1,025, were generally satisfied with the milk companies' policies. Interviewers on this assignment encountered no positive hostility. A report is also given on the milk-buying habits, brand-buying habits, reason for brand selection, reactions to suggested innovations, familiarity with milk industry, and the comparative ranking of factors influential in milk buying.

C.J.B.

- 534. Lipolysis in Raw Milk. Influence of Homogenization Temperature.** I. A. GOULD, Mich. State College, East Lansing, Mich. *Ind. Eng. Chem.*, 32: 6, 876-877. 1940.

Raw milk which was quickly heated to 70°, 105°, 115°, 125°, 135°, and 145° F. and homogenized immediately at 1500 lbs. pressure showed lipolysis in each case. Maximum fat splitting occurred within the temperature range 105°-125° F. with only a slight lipolysis at 145° F. Samples of milk treated in the above manner but stored at 35°-40° F. for 72 hours underwent much greater lipolysis than samples not stored but the trend of results was the same.

B.H.W.

- 535. The Cause and Control of Rancid Flavor in Milk.** N. P. TARASSUK, Univ. of California, Davis, Calif. *Can. Dairy and Ice Cream J.*, 19: 3, 32. 1940.

Rancidity in milk is the result of the hydrolysis of milk fat by a lipolytic enzyme. This enzyme may be secreted into the milk by the cow or may come from bacteria growing in the milk. Lipase may be present in milk but may not be active. It may be activated by shaking or homogenization or by temperature manipulation. It was found possible to produce at will either a milk high in natural lipolytic activity or one free from it by merely changing from a dry feed to a green feed. Lipase action results in the lowering of surface tension and this characteristic may be used as a means of determining rancidity. Heating of the milk results in inactivation of the lipase.

O.F.G.

- 536. Fluid Milk vs. Canned Milk.** C. W. PIERCE, Dept. Agr. Economics, Pennsylvania State College, State College, Pa. *Milk Dealer*, 29: 10, pp. 35, 63-64. July, 1940.

Statistics are given which show that the use of evaporated milk has increased rapidly in the United States. It is further shown that with the

increased use of evaporated milk there has been a gradual widening of the spread between the retail prices of fresh and evaporated milk. The author points out that a study of sales of milk from retail stores in New York City indicates that in low income areas and to a lesser extent in medium income areas there is a very definite relationship between the retail price differential and the sales of the two products.

The author states that: "The only logical conclusion is that an adjustment will have to be made in both the farm price and the distributor's margin if fresh milk is to compete effectively with evaporated milk."

C.J.B.

- 537. Preservation of Devonshire or Clotted Cream.** N. S. GOLDRING AND MAX E. MORGAN, Division of Dairy, Agr. Exp. Sta., State College of Washington, Pullman, Wash. Milk Dealer, 29: 10, pp. 32, 67-69. July, 1940.

From a study of the application of the frozen pack method to the preservation of Devonshire cream the authors concluded that: "Since it is not a general practice to hold frozen foods more than six to eight months and our experiments show that Devonshire cream can be held for approximately these periods, it may be definitely concluded that clotted or Devonshire cream is quite suited to the frozen pack method of preservation."

C.J.B.

- 538. Enzymes and Other Substances as Antioxidants in Milk.** D. H. NELSON AND C. D. DAHLE, Dairy Dept., Pennsylvania State College, State College, Pa. Milk Dealer, 29: 10, 41-55. July, 1940.

Details are given of experimental work to determine the efficiency of enzymes and other substances as antioxidants in milk. This work is summarized as follows:

Certain substances were found to inhibit the development of the copper-induced oxidized flavor in milk. Results obtained with pure ascorbic acid agree with the reports of other investigators that it inhibits the development of the off-flavor, and the ascorbic acid itself practically disappears before an oxidized off-flavor appears. However, when fresh tomato juice or fresh orange juice is added to milk, the protection is greater than would be expected from the amount of ascorbic acid which they contribute. Oat flour was also found to inhibit the development of the copper-induced flavor even after the ascorbic acid, which it did not protect, had disappeared. Therefore, a copper-induced flavor can appear only when practically all of the ascorbic acid has disappeared but will not necessarily develop in the absence of ascorbic acid, especially when certain water-soluble antioxidants are present.

Citric acid failed to exhibit antioxidative properties when added to milk, although other investigators have found it effective in lard.

Addition of very small amounts of trypsin and steapsin preparations to milk was found to inhibit the development of copper-induced oxidized flavor. Since pepsin exhibited no antioxygenic properties, and since steapsin exhibited greater protective powers than did trypsin, it is believed that the beneficial effect of trypsin and steapsin is due to their action on the fatty material rather than to their action on the protein fraction of the milk or on the oxidation-reduction potential. Several attempts to destroy entirely the activity of steapsin by heat or by copper, were unsuccessful.

Gum guaiac exhibited antioxygenic properties in milk when used in much smaller concentrations than any of the antioxidants used. This antioxidant is very interesting because it is insoluble in water and only very slightly soluble in fat. This suggests that certain antioxidants may not need to diffuse into the substrate. Furthermore, since these small concentrations of gum guaiac do not inhibit the oxidation of ascorbic acid, they would not be expected to influence the oxidation-reduction potential.

Crude sugar was also found to inhibit the development of copper-induced flavor.

C.J.B.

**539. A Rapid Phosphatase Test. Adaption of Scharer's Modification to Pasteurizing Conditions in Great Britain; B. A Study of Factors Influencing the Reliability of the Test.** R. ASCHAFFENBURG AND F. K. NEAVE, National Institute for Research in Dairying, Univ. of Reading, Reading, England. *J. Dairy Res.*, 10: 485-497. 1939.

A modification of Scharer's "10 minute field test" said to be applicable to British pasteurization conditions is described. The test requires less than 1½ hours for completion.

Experiments indicated a higher than normal phosphatase content in milk from cows suffering from mastitis both raw and after pasteurization. Unless more than 50 per cent of the cows contributing to a large bulk of milk are infected with mastitis it was found that this was unlikely to affect the results of the test.

S.T.C.

**540. Kay and Graham's Phosphatase Test. A. Modifications in Technique; B. Effect of Bacterial Growth.** F. K. NEAVE, National Institute for Research in Dairying, Univ. of Reading, Reading, England. *J. Dairy Res.*, 10: 475-484. 1939.

It is suggested that the technique of the phosphatase test be modified as follows:

1. By the addition of sodium hexametaphosphate before placing the tubes in the boiling water bath. This makes it possible to eliminate the final filtration.
2. Avoid direct sunlight during that part of the test in which Folin's reagent is in use.

The pH of the milk samples tested was found to vary naturally before pasteurization from pH 6.5 to 6.9 without affecting the validity of the test.

The test was unaffected by the presence in milk of bacteria which produce reducing substances or which hydrolyze the substrate (sodium phenyl phosphate) unless the organisms are present in very large numbers.

S.T.C.

## PHYSIOLOGY

- 541. H Ion Concentration of Various Fluids of the Genital Tract of the Cow.** HENRY A. LARDY, W. D. POUNDEN, AND PAUL H. PHILLIPS, Dept. of Biochemistry and Veterinary Science, Univ. of Wisconsin. Proc. Soc. Exp. Biol. and Med., 44: 517. 1940.

A study of the pH of the genital secretions of the cow showed the vagina to be slightly acid during diestrus (6.4) while it was slightly alkaline during estrus. The fluids of the cervix during estrus were slightly alkaline (8.3) while the fluids present in the uterus during estrus were slightly acid (6.8).

R.P.R.

- 542. Effect of Volume Used for Injection in Micro-Assay of Prolactin.** ROBERT W. BATES AND OSCAR RIDDLE, Carnegie Institution of Washington. Proc. Soc. Exp. Biol. and Med., 44: 505. 1940.

Results were presented which indicate the importance of utilizing a constant volume of fluid when assaying the lactogenic hormone by the local crop-sac method. A minimum dose of prolactin for stimulation of the crop gland in 0.05 ml. has its effectiveness increased four fold when diluted to 0.50 ml.

R.P.R.

## MISCELLANEOUS

- 543. Influence of Government Activities upon the Dairy Industry.** W. A. WENTWORTH, Dairy Industries Committee, Washington, D. C. Milk Dealer, 29: 9, 118-125. June, 1940.

A discussion is presented of the effect of government activities, such as the Agricultural Adjustment Administration, Wagner act, the wage and hour law, and reciprocal trade agreements, on the dairy industry.

C.J.B.

- 544. Water Conditioning for Dairy Plants.** G. A. RICHARDSON, Univ. of California. Can. Dairy and Ice Cream J., 19: 4, 38. 1940.

The salts of calcium and magnesium are directly responsible for the majority of the troubles arising from the use of untreated water such as the formation of scale, sludge and milk stone. Softening water is the render-

ing of the calcium and magnesium unavailable for the formation of insoluble soaps or precipitates. This may be accomplished by (1) distillation, (2) heating raw water followed by settling and filtration, (3) lime treatment, (4) treatment with lime and soda, (5) base exchange, and (6) addition of alkalies such as caustic soda, soda ash, trisodium phosphate, sodium metasilicate, tetrasodium pyrophosphate, sodium bexametaphosphate and sodium tetraphosphate. Boiler water may be softened either by treatment with lime-soda or by base exchange. Water for condensers should be treated so as to keep the calcium salts in solution rather than to precipitate them. Water for washing operations should be softened by base exchange or treatment with some of the phosphates mentioned above. These phosphates act by the prevention of precipitates.

O.F.G.

**545. Water—Taste—Odor—Color.** A. V. MOORE. *Can. Dairy and Ice Cream J., 19: 4, 52.* 1940.

One of the prime factors involved in the establishment of a dairy enterprise is having an adequate supply of clean water. A water-lubricated pump is the most satisfactory. A potable water is not necessarily satisfactory for dairy use. Damaging flavor defects of butter are often traced to the water used in washing. Unsatisfactory sediment tests of certain dairy products may be traced to the water used in processing. Milk stone may not be directly due to water but it is aggravated by certain types of water. Cream feathering may be due to the mineral content of water.

O.F.G.

**546. Two-Metal Contact Corrosion. Its Causes and Prevention.** H. E. TEPEL, Chief Engineer, Adalet Mfg. Co., Cleveland, Ohio. *Milk Dealer, 29: 10, pp. 36, 72-73.* July, 1940.

A discussion is given of the effect of galvanic action (electrolysis) on the corrosion of metals. Where piping systems transmitting electrolytes such as tap water, brines, and other solutions are made up of dissimilar metals, it is necessary to use insulated couplings in order to prevent the rapid disintegration of one of the metals.

C.J.B.

**547. Cutting Production Costs through Planned Work Schedules.** HANS EDEL, Gehl's Guernsey Farms, Milwaukee, Wis. *Milk Dealer, 29: 9, pp. 32-33, 59.* June, 1940.

The author presents a "working and relief schedule" for medium-sized plants. This schedule is made up weekly and shows the work of each man for the week, his time off, who is to relieve him while off or in case of accident. It also shows the number of hours worked in the various departments of the plant.

C.J.B.

**548. Good Water—a Necessity in the Dairy Products Plant.** K. G. WECKEL, Univ. of Wis., Madison. Nat. Butter and Cheese J., 31: 7, 14. July, 1940.

All water in a dairy plant should preferably be of "drinking water" quality although re-used, lake, pond or river water may be used for economical reasons. All water used for rinsing equipment, washing of cheese curd or butter, standardization of condensed milk products, or in the making of fruit ices or fruit drinks should be free of undesirable organisms. The presence of foreign materials such as iron, copper or brass from equipment or metal "chore boys," sludge, grit and calcareous sedimentary accumulations, rubber or particles from disintegrating hose, oil contamination from water-pumping equipment, all may cause defects in dairy products or difficulties in cleaning operations. The mineral and organic matter of water varies with the season of the year, geologic source and availability of the water. A table shows the maximum range in concentration of constituents observed in the water supplies of 100 American cities. The metallic constituents may cause off-flavors in dairy products while "hardness" may affect undesirably the protein stability during sterilization of evaporated milk, feathering of cream in coffee, quality of casein used in glues, paints, plastics and paper sizing and may increase deposition of milk stone. Hard water tends to increase labor costs, consumption of soap and alkali, cleaning problems and problems of boiler management. Plenty of water available makes labor more efficient and aids in quality control. Dairy plant operators should appraise their water supplies in terms of costs of obtaining them, costs of using them, their bacteriological, chemical and physical characteristics and their influence on products and plant efficiency. Well and pump engineers, water engineers and chemists should be consulted for advice on efficiency of pump installations and purity of water supplies.

W.V.P.

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## ABSTRACTS OF LITERATURE

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Columbus, Ohio

## MILK AND MILK PRODUCTS

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National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS TO APPEAR IN THE  
JOURNAL OF DAIRY SCIENCE

### 549. Avenized versus Standard Parchment for Wrapping Print Butter.

W. B. COMBS, S. T. COULTER AND DANA W. WHITMAN, University of Minnesota, St. Paul.

Samples of print butter from a large number of churningings were wrapped in standard parchment wrappers and wrappers that had been treated with oat flour (Avenex). The butters were stored for varying periods. The surface deterioration was measured organoleptically and by means of the fat aldehyde test. The results indicate that parchment paper treated with oat flour had a very slight effect in retarding the deterioration of the surface of butter. The treated parchment proved of most value when used on butter made from neutralized cream and of a "90" score.

### 550. Some Factors Affecting the Stability of Certain Milk Properties.

IV. Interrelation of Certain Metals and Metallic Ions and the Development of Oxidized Flavor in Milk. O. F. GARRETT, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

Contamination of milk with copper or ferrous iron is known to catalyze the oxidation reaction which produces oxidized flavor in milk. When divalent manganese was added to milk in molar concentration equal to or greater than either copper or iron the development of oxidized flavor was completely inhibited or greatly retarded for periods up to 96 hours. The manganese had a similar effect when strips of copper metal were placed in the milk. Pieces of manganese metal acted in a manner similar to the manganese salt.

Divalent manganese added to milk after the development of copper-induced oxidized flavor had begun arrested further development of the flavor. Trivalent aluminum ( $AlCl_3$ ) ions had no retarding effect on the development of copper-induced oxidized flavor.

The addition of manganese to uncontaminated or copper-contaminated milk had no effect on the oxidation rate of reduced ascorbic acid nor on the magnitude of the oxidation-reduction potential.

### 551. Some Factors Affecting the Stability of Certain Milk Properties.

V. A Comparison of Seven Different Roughages on the Color and Flavor in Milk. O. F. GARRETT, R. B. ARNOLD AND G. H. HARTMAN, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

The results of three separate experiments are reported. The first experi-

ment showed that milk produced on molasses alfalfa silage was almost equal in yellow color to milk produced on spring pasture (6.3 lactochrometer units and 6.4 lactochrometer units, respectively), was slightly better in flavor score than pasture milk, and resisted equally well with pasture milk the development of copper-induced oxidized flavor.

The second experiment showed that dried citrus pulp impregnated with molasses and dried beet pulp were about equally poor in producing yellow color, flavor and resistance to the development of copper-induced oxidized flavor in milk. Both types of pulp were greatly inferior to molasses grass silage with respect to these factors in milk.

The third experiment showed that molasses grass silage and phosphoric acid grass silage were about equal in producing milk of high yellow color, good flavor and high resistance to copper-induced or spontaneous development of oxidized flavor. Both types of grass silage were definitely superior to corn silage with respect to those factors.

**552. The Time of Ovulation in Cattle.** C. L. COLE AND J. E. BREWSTER,  
Dept. of Animal Husbandry, Michigan State College, East Lansing,  
Mich.

The increased use of artificial insemination has brought about a greater need for definite information relative to the time of ovulation in cattle. The results reported in the literature relative to the time of ovulation are variable and incomplete.

This study was carried out on both dairy and beef cattle. Seventy-three rectal examinations were made on 47 cows. Nine cows were slaughtered immediately after ovulation to check the results obtained by palpation.

Ovulation was found, in all except three instances, to occur within the first day after estrus. One cow ovulated on two occasions before she went off estrus. Another cow ovulated 26 hours after the end of estrus. The average time of ovulation from the end of estrus was  $13.57 \pm 0.68$  hours.

No significant difference in time of ovulation was noted between breeds, types of cattle, or time of day. Heifers ovulated on the average of 3.04 hours sooner than did cows that had calved previous to these studies.

Both ovaries produced follicles with equal frequency and there was no apparent order in which they functioned in any one animal.

**553. The Relation of Certain Factors to the Drying of Whey Mixtures on the Atmospheric Drum Drier.** E. L. JACK AND A. J. WASSON,  
University of California.

When whey alone is dried on the double drum atmospheric drier, a gummy mass results that is difficult to remove from the machine and which hardens when cool so that grinding is necessary to put it into useable condi-

tion. For the formation of a continuous sheet of dry material it is necessary to add a film-forming substance to the whey. Various materials have been used, including skimmilk solids, either in liquid or dry form, and cereal products. This study has been concerned with the properties of different combinations of whey and drying agents which yielded a satisfactory sheet when scraped from the drum.

It was found that when liquid skimmilk was used as the film-forming material it required about one and one-half parts skimmilk solids to one part whey solids at low acidities to form a satisfactory sheet. This represents about one part milk protein to two parts lactose. As the acidity increases the amount of skimmilk solids required increases also. When condensed skimmilk was used approximately equal parts of skimmilk solids and whey solids in the mixture formed a satisfactory drying combination. Increasing acidity again required that more milk solids be used. Mineral acids gave substantially the same results as developed or added lactic acid. Ground cereal products were also used. Approximately one part cereal product to two parts whey solids gave satisfactory results. Those found to be useable were flour, corn starch, ground oats (sifted), and ground barley (sifted). The amount of cereals required was not much affected by different degrees of acidity. The lactose : nitrogen ratios and the pH relationships have been determined.

#### BOOK REVIEW

- 554. Industrial Microbiology.** S. C. PRESCOTT AND C. G. DUNN. 541 pages, illustrated, price \$5.00. Published by McGraw-Hill Book Company, New York, N. Y.

The authors have treated the subject of industrial microbiology from the standpoint of the investigation and control of those fermentations that are of industrial importance because of their end-products or their effect in altering the quality or composition of certain substrates such as foods.

The book is divided into four parts. Part I discusses the characteristics, methods of handling, and industrial applications of yeasts. It includes production of industrial alcohol; mechanism of the ethyl alcohol fermentation; the brewing, wine and distilling industries; commercial yeast manufacture; and production of glycerol and fat. Part II includes the acetone-butanol; acetone-ethanol; butyl alcohol-isopropyl alcohol; acetic acid; commercial lactic acid and propionic acid fermentations; as well as certain fermentations important in the food industry. Part III is devoted to molds; industrial fermentations in which molds are utilized; mold enzyme preparations; and production of fat by molds. Part IV reviews the microbiology of wood and textiles. Also included are two useful appendices, one on detergency, disinfection and sterilization, the other on treatment and disposal of industrial microbiological wastes.

Discussions which are well written throughout cover not only the established industrial fermentations, but also some of the more recently discovered fermentations that offer possibilities for industrial application in the future. Applications of industrial microbiology as related to the manufacture of sera and related products and to certain phases of agriculture and dairy manufacture are omitted. This is unfortunate, but the breadth of the subject presents an overwhelming task to anyone attempting a thorough treatment of all phases of industrial microbiology.

Throughout the book, both in the discussions and at the end of each chapter, numerous references are supplied to guide the reader to further information on specific processes and general reviews of the subject. These aid materially in providing a book that should prove valuable, particularly for courses in industrial fermentations or food technology and microbiology.

P.R.E.

## BACTERIOLOGY

- 555. Further Studies on Development of *Clostridium botulinum* in Refrigerated Foods.** F. W. TANNER, P. R. BEAMER AND C. J. RICKHER. Dept. of Bacteriology, Univ. of Illinois, Urbana, Ill. Food Res., 5: 4, 323. July-Aug. 1940.

It was found that samples of food artificially inoculated with strains of *Clostridium botulinum* and frozen, were not toxic when thawed and held at 5° C. (41° F.) for 14 days. Similar samples thawed and held at higher temperatures were in general toxic, particularly when the pH of the food was higher than 4.5. The authors state that frozen foods, if properly handled and kept frozen until used, should be as safe and as satisfactory as similar fresh foods.

F.J.D.

- 556. New Media for Bacterial Counts.** H. G. HARDING, Akron Pure Milk Co., Akron, Ohio. Dairy World, 19: 4, 28. Sept. 1940.

A brief discussion of the effects of the new media adopted by the A.P.H.A. on bacterial counts and on compliance with milk ordinances. The author stresses the fact that the milk industry and control agencies are less dependent on plate counts as quality indications since the use of special tests is becoming more general. Such tests as the methylene blue, the resazurin, the direct microscopic examination, laboratory pasteurization and the phosphatase test are mentioned.

F.J.D.

## BREEDING

- 557. Reproductive Efficiency in Dairy Cattle.** F. E. HULL, W. W. DIMMOCK, FORDYCE ELY AND H. B. MORRISON, Univ. of Kentucky, Lexington, Ky. Bull. 402, 28 pages. May 1940.

The breeding efficiency of the University of Kentucky dairy herd was

studied from 1900 to 1939, inclusive, in relation to health conditions and control measures. During the period 1900 to 1927, inclusive, there was no organized program for Bang's disease control or other diseases except tuberculosis. From 1928 to 1932, inclusive, there was an intensive health supervision program during which Bang's disease was eradicated. From 1933 to 1936, inclusive, the herd was free from Bang's disease. From 1937 to 1938, inclusive, trichomoniasis infection occurred. A total of 482 individuals was involved. A summary of the results is presented in the following table:

Period inclusive	Calving interval per cow	Breeding efficiency	Abortions in terms of pregnancy	Per cent of pregnancies calves born dead	Calves born alive but died in 6 months	Per cent of pregnancies that grew to maturity
	months	%	%	%	%	%
1900-1927	17.2	69.8	15.6	3.1	12.1	71.5
1928-1932	18.0	66.7	12.4	10.5	3.7	74.2
1933-1936	14.2	84.5	9.3	8.0	0.7	82.1
1937-1938	15.7	76.4	7.3	7.3	5.7	80.5
1939	12.7	94.5	2.6	10.3	11.8	76.9
The whole period	16.6	71.8	13.6	5.1	9.3	73.7

W.E.P.

##### 558. Directions for the Ascorbic Acid Therapy of Slow-breeding Bulls.

PAUL H. PHILLIPS, Dept. of Biochemistry, Univ. of Wisconsin, Madison, Wis. J. Am. Vet. Assn., 97: 165-166. 1940.

Subcutaneous injection of ascorbic acid caused marked recovery in 65 to 75 per cent of impotent bulls. Approximately 5 mg. ascorbic acid should be injected per kilogram of body weight every 3 or 4 days over a period of 5 or 6 weeks. One gram of ascorbic acid is dissolved in 5 ml. of a buffer solution. The buffer solution is prepared by dissolving 0.1 gram monobasic potassium phosphate and 0.4 gm. sodium phosphate (U.S.P. dried) in 50 ml. distilled water.

W.E.P.

## BUTTER

##### 559. The Relation of Carbon Dioxide Gas to the Keeping Quality of Butter. W. B. COMBS, Dept. of Dairy Husbandry, Univ. of Minn., St. Paul, Minn. Ice and Refrig., 97: 3. 1939.

This paper outlines the procedure to be used in an experiment to determine the value of an atmosphere of carbon dioxide for preventing the development of stale and oxidized flavors on the surface of high quality butter.

L.C.T.

**560. Iron and Copper Content of Butter.** G. M. MOIR AND E. D. ANDREWS.  
N. Z. J. Sc. and Techn., 21: 249A-265A. 1940.

In a new filtration method for estimating iron, 10 grams of butter are melted in 15-ml. centrifuge tubes; 1 ml. of 5 per cent Soda hydro-sulphite is added, shaken and left over night at 35-40° C. After shaking with 1 ml. of 20 per cent trichloroacetic acid the tubes are stood 30 min. at 40-50° C., centrifuged, and the melted fat siphoned off. The aqueous layer is shaken with 5 drops of 10 per cent sodium tungstate and the later filtered cold through papers just previously washed with 5 per cent nitric acid, the filtrate is shaken with 0.5 ml. of saturated pot. persulphate, followed by addition of 2 drops each of nitric acid and hydrogen peroxide. After adding 1 ml. of 30 per cent ammonium thiocyanate, all tubes including standards are filled up to the same level. To extract the color 2 ml. of amyl alcohol are added and shaken. Standards and blanks are prepared with all the reagents included. To clear the amyl alcohol layer, the tubes are cooled in ice-water prior to the final color comparisons. Many results thus obtained have been compared with those yielded by an improved dry-ashing method.

For copper 25 gram samples are melted at 40-50° C. and shaken with 0.5 ml. conc. hydrochloric acid, 5 drops of 3 per cent hydrogen peroxide, 2.5 ml. of 20 per cent trichloroacetic acid, and 5 drops of 10 per cent sodium tungstate. After keeping warm 30 minutes the samples are centrifuged and the fat layer sucked off. The tubers are cooled prior to filtering through papers freshly washed with acid. One washing of the precipitate with 5 per cent nitric acid is followed by others with 5 per cent trichloroacetic acid. To the filtrate are added 2 ml. of 20 per cent sodium citrate, a few drops of phenolphthalein, and sufficient strong ammonia dropwise to make alkaline. A few mg. of powdered sod. diethyl-dithiocarbamate are added to each tube, the volumes equalized, and 5 ml. amyl alcohol added. The tubes are shaken, allowed to stand three or four hours, and shaken again. When clear the amyl alcohol colors are compared with similarly prepared standards and blanks, using if possible a Klett colorimeter with a blue filter.

For copper the wet-ashing method of Williams as modified by Koppejan and Van der Burg has been further improved. In a large centrifuge tube 25 grams of butter are warmed with 8.ml. of glass distilled nitric acid. The water-bath is raised gently to near boiling-point and effervescence dispersed by stirring. After an hour or more the fat is removed by centrifuging and sucking off, followed by two similar treatments with 5 ml. of high-boiling petrol. The acid liquid is washed out into a 200 ml. Kjeldahl flask and gently evaporated almost to dryness. When cool, 2 ml. of pure sulphuric acid are added, and after further heating small amounts of conc. nitric acid are added as required; later a few drops of perhydrol may be required to complete the oxidation. The residue is washed into a large test-tube, neu-

tralized, and other reagents, citrate, etc., added as in the filtration method. The original paper contains useful details about purifying reagents, etc.

Author's Abstract.

### FEEDS & FEEDING

- 561. Silage from Hay Crops. Making It—Feeding It.** S. T. DEXTER AND C. F. HUFFMAN, Michigan State College, East Lansing, Mich. Circ. Bull. 173, 8 pages. July, 1940.

A popular treatment of the problems in preparing and feeding grass silage. W.E.P.

### ICE CREAM

- 562. Stimulating Carry-Home Sales of Ice Cream.** ANONYMOUS. Ice Cream Rev., 23: 12, 24. July, 1940.

An unlimited increase in the per capita consumption of ice cream is possible by stimulating carry-home sales. Suggestions given for increasing these sales include: giving the consumer a greater knowledge on the preparation of sundaes, etc., in the home; using insulated bags to keep ice cream hard under adverse conditions; the use of flat refrigerator-type packages; and point-of-sale advertising suggesting specifically the "carry home" idea.

J.H.E.

- 563. A Change in Vanilla Nomenclature.** ROBERT ROSENBAUM, David Michael and Co., Philadelphia, Pa. Ice Cream Rev., 23: 12, 52. July, 1940.

Conforming to international rules the U. S. Department of Agriculture has adopted *Vanilla fragrans* (Salisb.) Ames as its official technical name for the source of our commercial vanilla beans. For years the beans were referred to as *Vanilla planifolia* Andrews. This terminology is now to be dropped. This new designation may have a bearing on the commercial products now being offered on the world markets. For instance, there is a question as to whether Tahiti beans are truly *Vanilla fragrans* and whether their use in products can be labelled as "vanilla." J.H.E.

- 564. Factors Affecting Mix Viscosity.** A. J. HAHN, Dept. of Dairy Husbandry, Univ. of Illinois, Urbana, Ill. Ice Cream Field, 36: 2, 26, 34, 35, 36. Aug., 1940.

Defining viscosity as "the ability of a liquid to resist flow" the author points out the necessity of distinguishing between "apparent" and "basic" viscosities. He further states that "fluidity" is the ability of any fluid to flow without the application of an exterior force; while "plasticity" is

the ability of any liquid to flow only after the application of an external force.

A brief discussion is given of the influence of the following factors upon ice-cream viscosity: (1) temperature, (2) mix composition and (3) methods of processing and cooling mixes.

It is stated that the use of unsweetened frozen cream in mixes results in about the same viscosity as that obtained with the use of fresh cream. It is further claimed that mixes made with concentrated milk, vacuum roll, or spray skimmilk powder and evaporated milk will not vary much in viscosity, but atmospheric roller powder, superheated condensed milk and sweetened condensed skimmilk will result in an increase in mix viscosity. Increasing the stabilizer content has a greater influence on mix viscosity than increasing other mix components.

It is claimed that as long as the acid content is not great enough to precipitate the proteins the viscosity of the mix will decrease with increased acidity, and further, that calcium and magnesium ions increase viscosity, whereas citrate and phosphate ions ordinarily decrease mix viscosity.

It is stated that homogenization causes a marked increase in mix viscosity especially if it is accomplished at 120° F. to 140° F.

A table is given showing the influence of the various factors considered upon viscosity and whipping ability of mixes and body, texture and flavor of ice cream.

W.C.C.

**565. Ice Cream Sales Index.** ANONYMOUS. Spec. Bull. of the Statistical and Accounting Bureau, Int. Assn. of Ice Cream Manufacturers, Washington, D. C. July, 1940.

This publication contains an analysis of ice cream sales for the first four months of 1940. During this period the sale of ice cream in the United States was 4.35 per cent higher than for the same period in 1939. Canadian sales showed an increase of 17.89 per cent when sales for the same periods were compared.

A supplement to the bulletin contains the following data: (1) Ice cream production in gallons by months by states, 1938; (2) Percentage of ice cream production by months by states, 1938; and (3) Percentage of ice cream production by months by states—ten year average, 1929–1938. M.J.M.

**566. New Rulings on Ice Cream under the Food and Drug Law.** R. C. HIBBEN. Ice Cream Trade J., 36: 7, 14. 1940.

Five new interpretations from the Federal Food and Drug Administration regulating ice cream shipped in interstate commerce are presented. These rulings cover the labeling of "coated ice cream," "ice cream sandwich," and "retail pails and cartons." The common name of chocolate ice cream and the regulation on the color declaration on labels of ices and sherbets are also discussed.

W.H.M.

**567. Vanilla in a Changing World.** R. C. SCHLÖTTERER. Ice Cream Trade J., 36: 7, 10. 1940.

The effect of the European war upon the production, procurement and price of vanilla is discussed. It is pointed out that Madagascar, the great vanilla producing center, is a French possession and that its transfer to Germany might bring out new economic problems. Due to the present disturbance of shipping and foreign exchange the normal market indices of supply and demand have lost their importance, therefore prediction of price or supply in this country is not possible. The Mexican beans, while higher in price, usually follow the supply and demand curve of the Madagascar product.

W.H.M.

**568. Do Small Stops Pay?** VINCENT M. RABUFFO. Ice Cream Trade J., 36: 7, 8. July, 1940.

The Diamond Company with headquarters in Jersey City, New Jersey, is doing an annual ice cream distribution of \$400,000 and finds that the small stops pay if properly managed. The secret is in keeping the stops close together, keeping waste and expense at a minimum, and not attempting to furnish dealers with supplies other than ice cream cabinets and very closely related materials. Heavy merchandising campaigns are not carried on because the small type of accounts do not warrant it. The Diamond Company does not manufacture ice cream but only maintains storage houses for keeping the ice cream prior to distribution. The head of the company estimates that 70 per cent of his sales are in packages and novelties; about 30 per cent in bulk. He has approximately 1450 dealers to which deliveries are made.

W.H.M.

**569. The Use of Fruits in Ice Cream.** B. I. MASUROVSKY. Ice Cream Trade J., 36: 8, 31. August, 1940.

\*Some of the latest developments in the use of fruits in ice cream are discussed. New possibilities are suggested. The author cites a paper by Dr. D. G. Sorber<sup>1</sup> containing the following directions for packing fruit for ice cream purposes.

- “1. Select full flavored fruit of predetermined suitable varieties.
- “2. Precool as an aid to controlling oxidation.
- “3. Wash the fruit thoroughly.
- “4. Coarsely crush or puree fruit in such a way as to avoid beating air into the product.
- “5. Add a predetermined amount of sugar or syrup and thoroughly mix to further aid in preventing enzymatic alteration of flavor and color.

<sup>1</sup> “The Preparation of Frozen Fruit Pulp and Their Use in Ice Cream and Related Products,” by Dr. D. G. Sorber, U. S. Dept. of Agriculture. (Report of Proceedings of the 39th Annual Convention, Int. Assoc. Ice Cream Mfgrs., Production and Laboratory Council, Vol. 2, 1939.)

"6. Pack in tightly sealed enamel-lined tin cans, preferably closed under vacuum.

"7. Rapid freezing at sub zero temperatures.

"8. Storing at a temperature of 0 degrees F. or colder." W.H.M.

## MILK

**570. Crystal Form of Ice Packing Appeals to Shippers of Perishable Foods.** ANONYMOUS. *Ice and Refrig.*, 97: 341. 1939.

A brief article which among other things points out the advantages of crystal form of ice packing for milk crates. This crystal ice may be briquetted by use of 17 tons pressure. L.C.T.

**571. Installation of FlakIce Equipment.** ARTHUR ADAMS, FlakIce Corp., N. Y. and R. E. MILLER, York Ice Machinery Corp. *Ice and Refrig.*, 96: 281. 1939.

This paper consists essentially of a story of the FlakIce installation in the N. Y. Sheffield Farms Dairy Plant, but it is of general interest because it includes a description of the equipment and operating characteristics. The machines resemble in general appearance double drum milk dryers. They are cooled with brine at from 0 to 14° F. and 8 to 10 lbs. per sq. inch pressure. The usual temperature rise is 2° F. The brine is maintained at a pH of 7.5 to 8.0. The ice leaves the rolls at about 20° F. and the storage rooms or bins are maintained at not higher than 22° F. The hoppers are so arranged that a predetermined amount of ice can be deposited on each crate of milk. Six to twelve pounds are generally used. A list of advantages of FlakIcing is given. L.C.T.

**572. A Study of Concentration and Freezing as a Means of Preserving Fluid Whole Milk.** R. T. CORLEY AND F. J. DOAN, Pennsylvania State College, State College, Pa. *Food Res.*, 5: 4, 369. July-Aug., 1940.

High temperature pasteurization (180° F. (82.2° C.) for 15 minutes) of fluid milk before condensation, homogenization and freezing retarded oxidation, lessened the tendency toward irreversible coagulation of protein and increased the possible storage period in the frozen condition as compared with low temperature pasteurization (145° F. (62.8° C.) for 30 minutes). Homogenization after condensing proved more effective in stabilizing the milk than when applied before condensing. Any copper contamination invariably caused tallowy flavors during storage. A storage period up to 15 weeks was possible. The reconstituted stored milk exhibited higher *in vitro* digestibility than similar normal milk. F.J.D.

- 573. Rancidity—Its Effects and Control.** K. G. WECKEL, Dept. of Dairy Industry, Univ. of Wisconsin, Madison, Wis. *Dairy World*, 19: 4, 16. Sept. 1940.

A brief discussion of the chemistry and biology of rancidity (hydrolytic) in milk and some milk products with descriptions of methods of measuring lipolytic activity and means of controlling the reaction. F.J.D.

### PHYSIOLOGY

- 574. Artificial Insemination.** C. L. COLE, Michigan State College, East Lansing, Mich. *Ext. Bull.* 207, 4 pages. June, 1940.

A brief consideration of the advantages and limitations of artificial insemination together with methods of application and organization problems. W.E.P.

- 575. Influence of Uterine and Ovarian Nerves on Lactation.** JOHN S. LABATE, Depts. of Anatomy and Obstetrics and Gynecology, New York Univ. *Endocrinology*, 27: 342. 1940.

An attempt was made to demonstrate the role played by the autonomic nerves supplying the ovaries, uterus, and hypophysis in the initiation and maintenance of lactation. Three control rabbits were bred and on the 25th day of gestation a Caesarian section was performed. The onset and duration of lactation was noted. Two rabbits were sympathectomized by removing all the known sympathetic pathways to the uterus, tubes and ovaries. They were then bred and treated as the control rabbits. Section was performed 27 and 32 days following sympathectomy. No difference in the onset and duration of lactation was observed between the two groups and both groups showed normal reproductive instincts. R.P.R.

- 576. Experimental Superfecundity with Pituitary Gonadotropins.** HERBERT M. EVANS AND MIRIAM E. SIMPSON, Institute of Experimental Biology, Univ. of California. *Endocrinology*, 27: 305. 1940.

Female rats from 26 to 34 days of age were injected with various levels of the follicle stimulating hormone alone and in conjunction with the principle in human pregnancy urine. Animals were usually sacrificed 12 or 22 days after breeding and implantation sites were counted. Supernumerary implantations were produced by the injected gonadotropins. The maximum number was 34, the average number was 17 which exceeded by at least 7 the number of implantation sites observed in normal rats. It was noted that a surprising number of embryos perish and undergo intrauterine resorption and that instances of prolongation of the span of gestation were common. R.P.R.

## MISCELLANEOUS

- 577. Trained Employees Build Good Will.** FRED E. KUNKEL. *Ice Cream Review*, 23: 12, 58. July, 1940.

Foremost Dairies, of Jacksonville, Florida, believe very strongly in the importance of good customer contacts. This article gives the plan the company has worked out to make employees realize that their contact with customers either helps or hinders their business.

J.H.E.

- 578. The Theory of Atmospheric Cooling Tower Operation.** F. F. STEVENSON, *Ice and Refrig.*, 98: 4, 273; 98: 348. 1940.

This paper consists of a technical discussion of the operation of cooling towers. The relationship of wet and dry bulb temperatures to the operation and size of cooling towers is explained. The various types of cooling towers are described. Factors affecting the performance of the towers are discussed.

L.C.T.

- 579. Notes on Corrosion Control in Refrigeration Condensers.** K. M. HOLADAY, Chemical Eng., Anheuser-Busch Inc. and A. VON GONTARD, Vice Pres. and Chief Eng., Anheuser-Busch Inc. *Ice and Refrig.*, 98: 286. 1940.

In this paper the authors report on the results of experimental work in connection with corrosion of condensers. The electrolytic and galvanic system of corrosion prevention is discussed, and it is shown that neither one has been effective to date, although the experiments have not yet been concluded. The use of sodium silicate in dosages sufficient to impart 8 parts of silica per million of water has not been found very effective. Further experiments under controlled pH conditions are desirable. The carbonate balance system showed considerable promise. The average requirement was approximately 0.10 lb. caustic soda per ton of refrigeration. A pH between 9.2 and 10.1 is most desirable. The use of paint has value if properly selected and applied. Some differences were noted in the rate of corrosion of various materials used in the construction of the pipes.

L.C.T.

- 580. Cost of Operation and Maintenance of Diesel Engines in Refrigeration Plants.** J. R. WATSON. *Ice and Refrig.*, 97: 143. 1939.

The author points out the importance of a good operating engineer for keeping costs low. In his discussion he shows the differences in cost per ton of ice resulting from the use of various drives, e.g., V-belt, flat belt, electric drive using direct connected generator and direct connected synchronous motor, for electric drive using direct connected generator and belted synchronous motor, and for electric drive using direct connected generator and belted induction motor.

A table giving Diesel operating costs for four ice plants follows:

	Tons ice	Fuel oil cost	Lub. oil costs	Cost per ton
1	90	4½¢	50¢	23.6¢
2	34	4½¢	50¢	23.4¢
3	28	4½¢	50¢	23.6¢
4	23	6¢	60¢	27.0¢

L.C.T.

**581. Some Problems in the Preparation, Processing and Distribution of Frozen Food Products.** W. E. GUEST, W. E. Guest and Co., Chicago. *Ice and Refrig.*, 96: 339. 1939.

This paper consists of a rather extensive abstract of a talk presented at the Dairy Manufacturers Conference at the University of Wisconsin, March 15, 1939.

Information on the preparation of vegetables, fruits, poultry, and meats for quick freezing are given. A brief discussion of packing and packaging material is included. Short descriptions of the direct contact, cold air circulation, brine or sirup spray, and immersion methods of quick freezing plant is estimated at \$25 or \$35 per pound of product per hour. A plant to handle 2000 pounds of product per hour would cost from \$50,000 to \$70,000.

L.C.T.

**582. Business Factors Affecting the Use of Cold Storage Lockers in Illinois.** E. N. SEARLS, Univ. of Illinois, Dept. of Agr. Econ. *Ice and Refrig.*, 96: 249. 1939.

A brief history of the development of locker plants indicates that the first known locker plant was installed by a creamery in Crete, Nebraska, in 1910. A creamery in Walla Walla, Washington, built a locker room in 1927. An independent creamery in Minnesota built a 48 locker plant in 1924 but the second plant in Minnesota was not started until 1935. In 1938 it was estimated that there were 2,000 locker plants operating in the U. S. F. A. Gouglar, of the Illinois Agricultural Association, 608 South Dearborn Street, in a survey made in 1938 of 13 Illinois locker plants showed that the total cost per locker ranged from \$22.60 to \$35.98. The average cost was \$30.33. A list of expense items is included. Many of these may be overlooked by the average owner. Factors involved in the management of a locker plant are listed. Additional suggestions for attaining successful operation include:

1. Securing an adequate amount of capital.
2. Locating the plant in a territory that justifies its existence.
3. Providing an operating income sufficient to meet operating expenses.

4. Setting up an accounting procedure that shows an accurate picture of the business operations.
5. Providing an informed and intelligent management.
6. Keeping directors informed monthly of the operations of the business.

L.C.T.

- 583. Building Complete Refrigerated Locker Systems.** GEO. C. FOERSTER, Mgr. Electric Dept., The Amana Society, Amana, Iowa. *Ice and Refrig.*, 96: 161. 1939.

The article is of interest not only because of the organization involved but also because of the floor plan which is included, as well as construction details which are given. Neglecting some of these details may mean the difference between satisfactory operation and dissatisfaction. L.C.T.

- 584. Refrigeration as Applied to Air Conditioning.** JOHN R. HERTZLER, York Ice Machinery Corp. *Ice and Refrig.*, 96: 105. 1939.

A brief description of electric, gas, coal, and oil operated units is included. Advantages and disadvantages are briefly listed. L.C.T.

- 585. Maintaining Refrigerating Plant Efficiency.** H. L. LINCOLN, Gen. Plant Mgr., Union Ice Company, San Francisco, Calif. *Ice and Refrig.*, 98: 368. 1940.

The author presents a convenient check sheet for making monthly operating comparisons. In making comparisons of one plant with another it is, of course, important to take into consideration such factors as water and air temperatures as well as any other items which might make a difference in operating conditions. Adjustments must therefore necessarily be made.

Graphs are included to show refrigeration required in ice storage under various conditions, ratio of tons of refrigeration to tons of ice where different raw water temperatures are used, refrigeration requirements for cold storage with varying outside temperatures, and a graph showing KW-hrs. per ton of ice at 20 lbs. suction pressure used by a belt-driven horizontal ammonia compressor when the temperature of the water to the condenser varies and when different raw water temperatures prevail. L.C.T.

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## ABSTRACTS OF LITERATURE

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International Association of Milk Dealers	The Royal Technical College, Copenhagen, Denmark
National Institute for Research in Dairying, Reading, England	United States Department of Agriculture
New York Association of Dairy and Milk Inspectors	

## ABSTRACTS OF LITERATURE

### ADVANCE ABSTRACTS OF REPORTS TO APPEAR IN THE JOURNAL OF DAIRY SCIENCE

- 586. Relationship of Curing Temperatures to Quality of American Cheddar Cheese.** H. L. WILSON, S. A. HALL, AND WM. T. JOHNSON, JR., Division of Dairy Research Laboratories, Bureau of Dairy Industry, U. S. Department of Agriculture.

A report of a study to determine the effect of curing temperatures on the quality of American Cheddar cheese in which one hundred and sixteen vats of milk of good quality were made into cheese, and the four duplicate cheeses made from each vat were stored as follows: One was held for 6 months at 34° F.; one for 3 months at 50° plus 3 months at 34°; one for 6 months at 50°; and one for 3 months at 60° plus 3 months at 34°.

The following results were obtained:

In the group stored for 3 months at 50° plus 3 months at 34° F., and also in the group stored 6 months at 50°, the majority of the cheeses were better in quality than the duplicates (or controls) that were stored 6 months at 34°. But in the group stored 3 months at 60° plus 3 months at 34°, the majority of the cheeses were poorer in quality than the duplicates stored 6 months at 34°.

Of the 116 cheeses that were stored 6 months at 50° F., about 80 per cent had a score of 92 or more at the end of the storage period and the other 20 per cent had a score of less than 92. Of the high-scoring group, 81 per cent were better in quality than, or as good as, the duplicate that was stored 6 months at 34°; whereas, of the lower-scoring group, only 26 per cent were better than, or as good as, the duplicate stored 6 months at 34°.

From these data, it appears that if a cheese is destined as a result of some inherent defect in the making process to have a score below 92, it will have a relatively higher score if it is held at 34° F. than if it is cured at 50°.

When cheese is held 6 months at 34° F., the percentage that will score 92 or better will be about the same whether the moisture content is above or below 38 per cent. When cheese is cured at any of the three higher temperatures used in this experiment, however, the percentage scoring 92 or better will be from 5 to 15 per cent in favor of the low-moisture cheese.

Cheese made from milk of good quality and by methods which insure cheese of good quality can be cured at temperatures as high as 50° F. with reasonable certainty of developing a clean and characteristic Cheddar flavor.

From past observations, cheese made from bacteriologically poor milk or so manipulated that there is a tendency to develop acid, bitter, or other off-flavors, should be stored at 34° F. in order to retard the development of these defects as much as possible.

- 587. A Technique for Perfusing Excised Bovine Mammary Glands.**  
W. E. PETERSEN, J. C. SHAW, AND M. B. VISSCHER, University of Minnesota.

Details are given for a suitable apparatus that will produce the necessary pulsations and the desired blood pressure, and has provisions for aeration of the venous blood. Care in excising and handling the mammary gland as well as the general features involved in conducting a perfusion experiment are discussed.

W.E.P.

- 588. Some Factors Involved in Efficient Milking.** KENNETH MILLER AND W. E. PETERSEN, University of Minnesota.

Data are presented to show the effect upon milk and fat production of lengthening the interval between milking and stripping; stimulating the cow to let down the milk sometime before the milking begins and increasing the length of time involved in the milking process. Delaying the time when cows are stripped following the milking machine has little or no effect upon milk and butterfat production. Stimulating the cow to "let down" milk 20 minutes before milking decreased both milk production and fat percentage and had a tendency to "dry off" cows. Lengthening the time required for milking had the same effect as stimulating the "let down" before the milking begins. The observed effects are tentatively explained as being due to a dissipation of the oxytocic principle before the milk is emptied out of the alveoli and ductules of the gland.

W.E.P.

- 589. The Relationship of Ascorbic Acid to Reproduction in the Cow.**  
PAUL H. PHILLIPS, H. A. LARDY, P. D. BOYER, AND GEORGE M. WERNER, Departments of Biochemistry and Dairy Husbandry, University of Wisconsin, Madison.

The results of these experiments have shown (1) that the ascorbic acid content of plasma averaged 0.39 mg. per cent for cows of all breeds studied, (2) that there is a difference between breeds with the Holstein lower than the Guernsey, (3) that there is a higher concentration of ascorbic acid in the plasma of the cow in mid to late estrus than there is in anestrus, (4) that generally speaking there is no difference between good and poor breeders in their peak concentrations of ascorbic acid during estrus, (5) that the subcutaneous ascorbic acid therapy of "hard to settle" cows results in a positive response in 60 per cent of the cases treated, (6) that ascorbic acid therapy does not correct cases of cystic ovary or other anatomical abnormalities, and (7) that  $\alpha$ -tocopherol proved ineffective in restoring tone to a toneless uterus.

From these researches the conclusion is evident that ascorbic acid is intimately associated with the early phases of the reproductive processes and

it can be successfully used as a therapeutic measure in treating certain types of sterility in the cow.

## BOOK REVIEWS

- 590. Judging Dairy Cattle.** E. S. HARRISON, Cornell University. March, 1940. 132 pages. Price \$2.75. John Wiley and Sons, Inc., New York.

This book makes a valuable addition to the literature on dairy cattle breeding and judging. It is beautifully illustrated. The photographs used are those produced by H. A. Strohmeyer, Jr., and J. T. Carpenter, Jr. The subjects were well selected and the photography is excellent. The author relies on the pictures to explain his points and to demonstrate the characteristics under discussion. The subject matter is brief and to the point. This book should be of interest to teachers, dairy cattle judges and breeders.

The author introduces the main theme of the book with a brief statement of his philosophy about dairy cattle breeding and the importance of type in a constructive breeding program. He makes it clear that he believes that first and foremost a dairy cow must produce large amounts of milk and fat year after year. He questions the value of short time records or even single lactations as a measure of a cow's ability. He feels that a great majority of the high producing cows which continue to produce year after year are cows of acceptable type. He draws these conclusions from his immediate experience with the Cornell University dairy herd and from a study of the production records of show cattle and the production records of cattle officially classified.

In chapter one the author reproduces the score cards and the true type pictures of the cows of the five dairy breeds. He discusses the value of the score cards and use that may be made of them.

He illustrates with photographs, in chapter two, the difference between desirable and undesirable heads and necks. The pictures are accompanied with a minimum of explanatory material.

Chapter three deals with the mammary system. Pictures are extensively used to show in great detail the udder, flank and milk veins, both side and rear views. They show the common udder faults, accompanied by brief explanations. In this chapter, two excellent udders are shown dry and in full milk flow. Here the author discusses the characteristics to be looked for in the dry and lactating udder.

In chapter four, the legs and feet come in for consideration. The author stresses the desirability of strong legs properly placed and set. The common faults, such as crooked hind legs, close hocks, overly straight hind legs and weak pasterns, are all well portrayed and described. Several pages are devoted to the need of giving feet the proper care. The author shows what happens when feet are neglected and allowed to get long and out of shape.

Several pictures are used to show just how a cow's foot should be trimmed. This is an especially good demonstration and shows the methods which all dairymen might well use.

Chapter five deals with the chest, chine, crop and shoulders. The author gives reasons for the desired characters and demonstrates effectively with photographs the correct and incorrect form. The photographs show the chest conformation and its relation to the set of the front legs.

Chapter six is devoted to reasons and placings. Here, pictures show front, rear and side views of several pairs of cows and the author gives his reason for the placing of each pair.

Chapter seven is given over to the problem of heifer judging. The author here spends considerable space explaining the characteristics to look for in the undeveloped udder. He emphasizes the need to consider the size, shape and placement of teats as well as udder attachment fore and rear. This chapter includes an interesting group of photographs of two noted show cows portraying their development from calfhood to maturity.

The last chapter deals with judging of bulls. The author recognizes the fact that the real value of a bull is determined by his get. In the show ring he feels that the bull of sharp angular type should be given preference since that corresponds to the type desired in cows. Here as elsewhere, the author uses numerous pictures to illustrate his meaning. C. L. Blackman

**591. Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists; 5th Edition.** Published by Association of Official Agr. Chemists, Washington, D. C. 757 pages, \$5.00.

This handbook of analytical procedures published about every five years is a compilation of tentative and official methods for the analysis of food and agricultural products. Analytical methods are proposed by a referee of the Association for acceptance as tentative methods after having been subjected to collaborative study in various laboratories. Tentative methods, after having been given further study and consideration may become, upon approval by the Association, official methods. The official methods are acceptable for court action; tentative methods are acceptable for court action in the absence of official methods for the same analysis.

The book consists of 40 chapters plus (25) tables, appendix and index. Chapters of particular interest to the dairy industry are those on beverages; malt beverages, sirups and extracts, and brewery materials; cocoa bean and its products; coloring matter in foods; dairy products; eggs and egg products; flavoring extracts; fruits and fruit products; metals in foods; oils, fats and waxes; sugars and sugar products; vitamins; waters; brine and salt.

New additions to the sections on Dairy products are the phosphatase

test (Gilcreas), the bioassay technique for the vitamin D line test, estimation of citric acid in malted milk powder, determination of mold mycelia in butter, and estimation of solids in milk by specific gravity measurements. Revision and extension was made of methods for detection of gums in cheese and the preparation of butter samples for estimation of moisture content. Tentative methods which have become official are: the estimation of fat in malted milk, and ash and total chlorides in cheese. Approximately 100 tests (primarily proximate analyses) for dairy products alone are detailed.

Tentative methods for estimating zinc colorimetrically, for determining the thiocyanogen number of fats and oils, and a micromethod for reducing sugars are included in other sections. Microbiological methods for examination of frozen egg products, and sugar are also included.

The new section on vitamins includes only assay methods for vitamin D in milk and in concentrates for poultry feeds.

Despite enlarged scope and increased material, the convenient size of this famous "Book of Methods" has been retained by economy of verbiage, without affecting its usefulness.

K.G.W.

## BACTERIOLOGY

592. **The Rosenthal Anaerobic Method.** A. A. MILES. *Lancet*, 239: 7. 1940.

When "technical" chromium powder was substituted for pure chromium powder in the Rosenthal method a gas was evolved which was bactericidal to some species of spore-bearing anaerobes and bacteriostatic to others. The toxic gas was believed to be hydrogen sulphide. J.F.C.

## BUTTER

593. **New Aids to Better Cream.** C. H. PARSONS, Swift and Co., Chicago, Ill. *Nat. Butter and Cheese J.*, 31: 8, 14. 1940.

It was observed that mixed lots of cream when held under adverse conditions tend to be nearer the quality of the poorest component rather than an average of all lots. A series of experiments which were conducted to simulate farm conditions show that the quality of cream is somewhat better maintained when containers of minimum surface area are used and when the cream is not stirred during the gathering period, rather than when held in the conventional cream can and stirred after each addition of cream. Investigations should be initiated to simplify and improve methods of maintaining quality of cream on the farm and should deal with such problems as cleaning and sterilization of separator and utensils, exclusion of extraneous matter, cooling methods, and design of cream containers. W.V.P.

594. What is the Cost of a Pound of Butter? G. M. PELTON, Swift and Co., Chicago, Ill. Nat. Butter and Cheese J., 31: 8, 10. 1940.

Every creamery operator should determine costs in his own plant. Kinds of costs and expenses are: procurement expenses, plant delivered cost and processing expense. These costs and expenses are broken down to show specific items which must be included in calculating cost per unit of production. Operators should figure test costs frequently to guard against unexpected changes and are shown how to make the calculations. No actual costs are given.

W.V.P.

595. Incubation Test as an Indication of the Keeping Quality of Butter. H. B. NAYLOR AND E. S. GUTHRIE, Cornell Univ., Ithaca, N. Y. Nat. Butter and Cheese J., 31: 9, 10. 1940.

Ten churning which were laboratory made under conditions to insure the best possible keeping quality and 28 churning from 4 creameries were subjected to the incubation test for keeping quality. Salted samples from each churning were tested for moisture and salt. Salted and unsalted samples were analyzed for total and caseolytic bacteria, pH and scored for flavor when fresh, after 7 and 14 days storage at 60°, after 3 months at 0° F. and after 10 days at 60° F. following the 0° F. storage period. Salted and unsalted butter made in the laboratory showed little or no flavor deterioration under any conditions. The incubation test predicted fairly well the keeping quality of the high grade butter. The pH of salted samples (9.5 per cent NaCl in the brine) was lower than the unsalted, probably due to the NaCl effect on the hydrogen ion. There was no correlation between either pH or bacterial content and keeping quality of high grade butter. The incubation test was quite reliable for predicting keeping quality of commercial butter samples. The salted samples kept best. Flavor and pH values on unsalted butter dropped during incubation both before and after cold storage but pH in salted samples did not change regardless of flavor changes. Only unsalted samples seemed to show deterioration in flavor due probably to caseolytic types of Gram-negative, rod-shaped organisms.

W.V.P.

596. Preparation and Care of Starters. Article 1. MICHAEL B. MICHAELIAN, Verley Products Corp., Chicago. Nat. Butter and Cheese J., 31: 9, 16. 1940.

This article defines bacteria, yeasts and molds and explains some terms commonly used in discussing starter cultures. The cause of aroma and flavor in starters is reviewed. The creatine test for acetyl methyl carbinol plus diacetyl and its application in judging starters are described.

W.V.P.

- 597. Factors Affecting Mold Mycelia Content of Butter.** W. L. SLATTER, Ohio State Univ., Columbus, Ohio. Nat. Butter and Cheese J., 31: 9, 50. 1940.

Representative samples of first and second grade creams were obtained from individual producers in central Ohio. Acidity, fat, mold, yeast and mold mycelia content were determined on each raw cream sample. Samples were neutralized, pasteurized, cooled and churned. Mold, yeast and mold mycelia content of the butter were determined. Mold mycelia contents of butter and the cream from which it was made are not closely related. From questionnaires filled out by each producer it was determined that butter with the lowest mold mycelia content is produced by large herds where cream is cooled, kept cold and delivered at least twice a week. W.V.P.

### CHEESE

- 598. Use of By-products in Making Cheese Spreads.** C. R. BARKER, Oak Park, Ill. Nat. Butter and Cheese J., 31: 8, 64. 1940.

Cheese processed with condensed or dried whey or condensed or dried skimmilk forms an excellent cheese food. Five to 10 lbs. of whey solids can be used per 100 lbs. of finished product; more whey solids tend to produce lactose crystals. Desirable spreading properties are produced with 42 to 44 per cent water. When skimmilk solids are used the casein builds up cheese body so that cream, butter or water must be added to give spreading properties. The pH of cheese mixtures should be adjusted to 5.5 to 5.9 with citric or phosphoric acids to avoid putrefactive spoilage. W.V.P.

- 599. Retarding Mold in Cheese.** ANONYMOUS. Nat. Butter and Cheese J., 31: 8, 12. 1940.

\* Frederick W. Miller, Jr., E. I. du Pont de Nemours and Co., conducted tests to show the effects of propionic acid, and sodium and calcium propionates on growth of mold on cheese cuts enclosed in cellophane. Half pound wedge-shaped pieces of cheese were first exposed to air contamination then immersed in the treating solution, drained, wrapped and sealed in 300 MST Cellophane bags, and finally stored at 58° to 60° F. and 90 per cent relative humidity. The results show that propionic acid is about twice as effective as the solution of salts. Mold growth is retarded by more concentrated solutions, longer immersion periods, shorter draining intervals before wrapping, tight fitting wrappers and limited amounts of contamination. Treatment in 8 per cent propionic acid for 15 seconds and a 4-minute draining period produces 18 to 21 days mold delay. Excessive concentrations or prolonged treatments with acid or salt solutions cause undesirable changes in color and surface flavor. W.V.P.

- 600. Process Cheese on a Small Scale.** C. R. BARKER, Oak Park, Ill. Nat. Butter and Cheese J., 31: 9, 15. 1940.

Prospective manufacturers should have a knowledge of bacteriology, chemistry, dairy husbandry, cheese manufacture and selling. Recommended pieces of equipment for a single story operation are a steam jacketed kettle, a large powerful grinder, an inexpensive filling machine to match the kettle in size, and a conveyor for boxes. W.V.P.

- 601. Fifty Years of Cheese Making.** C. F. DOANE, Salem, Oregon. Nat. Butter and Cheese J., 31: 1-9. 1940.

Information from fifty years of close contact with the cheese industry is reviewed in a series of 9 articles published in 9 issues. I (31: 1, 30. 1940) The granular type of cheese made 50 years ago has practically disappeared along with the curd sink and blade curd knife. The hot-iron test made development of the cheese industry possible and the whey separator made factories more efficient. II (31: 2, 30. 1940) High ideals of quality have been lowered by the sale of green cheese, by excessive yields and lack of payment for a superior cheese. The consumer does not recognize inferior quality in green cheese. III (31: 3, 56. 1940) Cheese making has been favored by climatic conditions in northern states. Pasteurization of milk has been introduced as a necessity in southern states and New Zealand but the value of the process can be questioned because of ripening difficulties. Process cheese lacks positive quality but sells because it is uniform, advertised, packaged and mild in flavor. IV (31: 4, 42. 1940) Good milk is necessary for good cheese. Helpful tests to measure quality are the curd test, gas tubes and methylene-blue test. Tillamook has made better returns because of high quality milk and this can be done anywhere in the United States not affected by long hot spells. Utensils, strainers and milking machines may contaminate milk but every factory patron must produce good milk and can do so easily at practically no extra expense. V (31: 5, 14. 1940) Acid development is commonly linked with desirable quality and control of fermentations in cheesemaking. But acid development should be restricted for best results. Even in control of gas, acid may really be less effective than commonly supposed. Low acid development in making lengthens the time of curing but improves cheese quality, particularly the flavor, and decreases chances of loss from excess acid. VI (31: 6, 36. 1940) Standards of composition compatible with good quality are fixed by law. It is the privilege and duty of skillful makers to control cheese composition. Such control can be attained through proper manipulations of acid and heat plus daily tests for moisture. Where correct standardization of fat is practiced there is no loss of quality. VII (31: 7, 12. 1940) Canning of natural cheese for curing retains volatile flavors both good and bad and the flavors of the cheese are thus intensified even

when a venting valve is used to release partially the pressure of gas formed during normal curing. A flavor called salty-acid or metallic in canned cheese is a permanent defect but putrid flavor and odor tend to disappear on exposure to air. Even good milk may produce cheese with some off flavors when it is ripened in cans. Pasteurized milk has been used to commercialize cheese canning but this treatment does not always produce the desirable flavors of raw-milk cheese. The salt-acid criticism may be due to the natural salts of milk rather than to bacterial changes. The addition of water to the milk removes some of the milk salts and the resulting cheese is improved in salt-acid flavor and produces gas for a shorter time in curing. The characteristic putrid flavor is eliminated when the canned cheese is ripened under a slight vacuum. The use of a square hoop and can to form and cure many small rectangular packages of cheese at moderate cost may be a desirable development but the plan of merchandising is not yet clear.

VIII (31: 8, 30. 1940) The Tillamook cheese industry is an unusual cooperative effort which has prospered because of a good manager, favorable climate, control of milk and cheese quality, careful grading and uniformity of product.

IX (31: 9, 12. 1940) Starter organisms help develop acid more rapidly in cheesemaking but may not control undesirable types of bacteria. Starter added to excess spoils Cheddar cheese eventually if not immediately; 0.5 per cent is more satisfactory than 0.75 per cent which is about the safe limit. Cheese makers should select starters carefully because of variations in the results attained with different cultures.

W.V.P.

## CHEMISTRY

602. **A Biological Assay of Riboflavin in the Liver of the Cow, Calf, Sheep, Lamb, and Hog.** O. B. SAFFRY, H. S. COX, B. L. KUNERTH, AND M. M. KRAMER, Kansas State College, Manhattan, Kansas. J. Nutrition, 20: 169-174. 1940.

The riboflavin content of beef, calf, lamb and pork livers was determined according to the Bourquin-Sherman method. The riboflavin values per 100 grams were as follows: Lamb-liver, 4950-5400 mg.; mutton liver 4350 mg.; calf liver, 3450-4350 mg.; beef liver, 2850-3450 mg. and pork liver 2700 mg. Liver samples purchased in the winter season were slightly higher in riboflavin than samples purchased in the fall.

C.F.H.

603. **The Effects of High Pressure on the Activity of Pepsin and Rennin.** JASON E. MATTHEWS, JR., R. B. DOW, AND ARTHUR K. ANDERSON, Dept. of Agr. and Biological Chem., and Dept. of Physics, Pennsylvania State College, State College, Pa. J. Biol. Chem., 135: 697. 1940.

The activity of pepsin and rennin decreased with pressure increase at

constant exposure time and was completely destroyed at pressures ranging between 5000 and 6000 kilos per sq. cm. Increase of exposure time at constant pressure likewise decreased the activity but to a lesser degree. Inactivation of pepsin and rennin by pressure depended strongly on the temperature. Loss of activity appeared to be dependent on certain buffers and the pH.

Both enzymes were heavily coagulated by pressure treatment at 10,000 kilos per sq. cm., a result that appeared to be the same as when the solutions were heated to 100° C. Although the product of denaturation by pressure appeared similar to that which had been denatured by heat the energy relationship is different in the two processes.

V.C.S.

## CONCENTRATED AND DRY MILK; BY-PRODUCTS

- 604. It Pays to Condense.** P. S. LUCAS, Michigan State College, E. Lansing, Mich. Nat. Butter and Cheese J., 31: 9, 36. 1940.

Practical, efficient equipment for concentrating small amounts of milk is now available at reasonable cost. Small milk plants should consider the use of such equipment to dispose of milk solids profitably. Omitting overhead, the cost of condensing 30 cans of skimmilk to 10 cans in a 3-foot pan were estimated to be \$0.79 for coal, \$0.12 for electricity, \$0.60 for water and \$1.13 for labor.

W.V.P.

## DISEASE

- 605. The Control of Streptococcic Mastitis in a Certified Herd.** O. W. SCHALM, Univ. of Calif., Berkeley, Calif. Cert. Milk, 15: 167, 11. March, 1940.

This paper presents the methods employed in eradicating streptococci mastitis from a certified herd. The program was based on the assumption that every cow shedding *Streptococcus agalactiae*, regardless of the condition of its udder, is a potential source of infection of the clean cows in the herd. Once a cow was classified as infected, it remained in that classification irrespective of subsequent findings.

W.S.M.

- 606. Tubercle Bacilli of the Bovine Type in Skin Lesions.** Editorial, Am. J. Pub. Health, 30: 551-552. 1940.

This editorial discusses an article by an investigator in Western Poland who finds that the bovine type is more frequent in skin tuberculosis than in any other organ of the body. *Lupus vulgaris* is the most frequent form of skin tuberculosis in Poland and it is estimated that there are some 25,000 cases of lupus in that country. The cattle in Poland have a rather high per cent of tuberculosis and in Warsaw 50 per cent of the market milk is contaminated with the tubercle bacillus.

M.W.Y.

607. **The Deterioration of the Bovine Udder in the Absence of Streptococci.** E. G. HASTINGS AND E. H. PETERSON. *J. Agr. Res.*, 60: 3. Feb., 1940.

Observations were made to determine the chemical composition and bacterial flora of the foremilk of 11 Holstein-Friesian animals for the first three lactation periods. The animals were isolated from other animals and fed rations typical for many dairy farms. The chlorine and catalase values were found to increase during a lactation period and also increased from one lactation period to the next. By the middle of the third lactation period the average percentage of chlorine and catalase was above the values usually considered normal. The results of the bacteriological studies supplied no explanation for the chemical changes in the milk. The udders of each animal remained free of streptococci and no other organisms were found consistently or to any great extent during the three lactation periods. The authors suggest that the causes of mastitis or of deterioration of the udder may be more complex than now supposed and indicate the need for more detailed and prolonged study of the subject.

W.J.C.

#### FOOD VALUE OF DAIRY PRODUCTS

608. **Nutritional Aspects of Milk.** W. E. KRAUSS, Ohio Agr. Exp. Sta., Wooster, Ohio. *Cert. Milk*, 15: 167, 3. March, 1940.

A discussion is given on the use of our newer knowledge of nutrition for making milk a better food than it already is.

W.S.M.

609. **Iron Utilization in Dogs on Milk Diets.** D. V. FROST, C. A. ELVEHJEM, AND E. B. HART, Dept. of Biochem., Univ. of Wisconsin, Madison, Wis. *J. Nutrition*, 19: 311-320. 1940.

The ratio of requirement of iron to copper is only about 5 to 1. Iron is absorbed and stored, but does not become available for hemoglobin building unless adequate copper is supplied.

Some degree of caution should be urged regarding the use of cobalt as a hematopoietic agent. Although in many instances small amounts of cobalt have appeared to stimulate hematopoiesis in dogs on milk diets, a clear-cut need for this element has not yet been established.

C.F.H.

610. **Ascorbic Acid Content of Goat's Milk and Blood: Influence of Ascorbic Acid and Injections and Diet.** M. S. RICHMOND, G. H. SATTERFIELD, C. D. GRINNELLS, AND W. J. DANN, Univ. of N. Carolina, Raleigh, and Duke Univ., Durham, N. Carolina. *J. Nutrition*, 20: 99-108. 1940.

Goats were fed a ration consisting of alfalfa or lespedeza hay and a grain mixture consisting of 4 parts of corn, 3 parts cottonseed meal, 2 parts wheat

bran and 1 part oats. The animals were turned to pasture in season. Blood and milk samples were taken at approximately weekly intervals.

The ascorbic acid in blood ranged between 0.6 and 0.8 mg. per 100 ml., and in milk between 0.5 and 2.0 mg. per 100 ml. There was no consistent relationship between the amount of ascorbic acid in the milk and that in the blood. The alternate feeding of a normal and an ascorbic acid-free diet to four goats indicated that the ascorbic acid of the blood is not closely dependent on the amount of ascorbic acid in the diet.

The injection of 1 or 2 gm. of ascorbic acid intraperitoneally resulted in a large increase in blood and urine ascorbic acid, and a slight increase in milk ascorbic acid.

C.F.H.

- 611. The Utilization of Calcium in Carrots, Lettuce and String Beans in Company with the Calcium in Milk.** J. B. SHIELDS, B. W. FAIRBANKS, G. H. BERRYMAN, AND H. H. MITCHELL, Div. of Animal Nutrition, Univ. of Illinois, Urbana. *J. Nutrition*, 20: 263-278. 1940.

Rats were used for experimental animals in a study of the relative availability of calcium from various sources. The results indicated that the commercial desiccation of milk does not appreciably impair the value of its calcium in the nutrition of growing rats. The calcium of milk was definitely better utilized than the calcium of fresh carrots, fresh lettuce and fresh green string beans, which was 85, 80, and 74 per cent respectively as available as the calcium of milk.

The steam cooking of carrots and the commercial canning of green string beans do not modify appreciably the value of these vegetables as sources of dietary calcium.

C.F.H.

## HERD MANAGEMENT

- 612. Raising Calves on Wire Floors.** H. H. TUCKER, Educational and Research Bureau for By-Product Ammonia, Columbus, Ohio. *Cert. Milk*, 15: 168, 9. April 1940.

This article describes in detail the construction of wire floors for the calf pen. The virtues for this new type of floor are, (1) the labor required to care for calves is greatly reduced over that normally required; (2) a great saving in bedding; and (3) an increased growth and development of the calves, and decreased losses due to death and sickness.

W.S.M.

## MILK

- 613. Receipts, Utilization, and Prices of Milk and Cream in Maine Milk Control Areas.** GEORGE F. Dow, Maine Agr. Exp. Sta. Bull., 399. March, 1940.

A presentation of developments in the public control of milk prices up

to July, 1939, an analysis of milk distributors' records submitted to the Maine Milk Control Board up to December 31, 1937, and a study of other readily available information pertaining to milk distribution in Maine. Twenty thousand, four hundred and twelve monthly reports of 927 distributors were included in the study of 29 areas, comprising receipts and disposal of 109,392,867 quarts of milk and 4,936,549 quarts of cream. A study of milk control operation in the state of Maine included in this bulletin is of interest because it is quite largely based upon the business of the producer-dealer and small dealer type rather than that of the large milk dealer. Average surplus in markets included in the study was 16.0 per cent for milk and 17.3 per cent for cream. A flat price plan was used for paying producers in the smaller markets where producer-distributors handled most of the milk. A classified price plan was used in eleven of the larger markets where large quantities of milk were purchased. The average price paid producers per quart of 4.0 per cent milk under a classified price plan was 5.9 cents for fluid milk, 3.9 cents for surplus milk, and 5.5 cents for all milk. The average price paid producers for heavy cream in most markets was \$1.75 per gallon. None of the markets had regulations providing for base ratings or equalizations of sales because the amount of surplus milk was relatively small. The average spread for distributing cream was three to six times higher than for milk, indicating relatively wide spreads and high profits per unit of cream sold.

L.M.D.

**614. Why Milk?—From the Standpoint of Human Health and Economics.** E. V. MCCOLLUM, Johns Hopkins University, Baltimore, Md. Cert. Milk, 15: 169, 3. May, 1940.

The importance of milk in the diet from the standpoint of proteins, minerals and vitamins is discussed. It is pointed out that nutritionists are gradually impressing upon the public that prevention and not cure of deficiency diseases is the object to be sought.

W.S.M.

**615. Conditions Influencing Cream Volume of Raw and Pasteurized Milk.** C. L. ROADHOUSE AND J. L. HENDERSON, Univ. of Calif., Davis, Calif. Cert. Milk, 15: 168, 7. April, 1940.

It was found that the rapidity and extent of cooling have the greatest influence on the cream layer volume of both raw and pasteurized milk. However, the temperature to which the milk is cooled to give the maximum cream layer is not the same for both kinds of milk. The greater cream layer volume in raw milk will be secured when it is cooled immediately to 50° F. after milking, bottled and then stored for several hours at 40° F. In pasteurized milk the greatest cream layer volume is secured when the milk is flash cooled to 40° F. or below and stored at 40° F. or below until the creaming has been at least partially completed.

W.S.M.

**616. Comparative Study of the Bacterial Flora of Grade A and Grade B Milk in New York City.** M. L. ISAACS AND M. NUSSBAUM, DeLamar Inst. of Pub. Health, Columbia Univ., New York, N. Y. Am. J. Pub. Health, 30: 9, 2-22. 1940.

A total of 1,130 samples of Grade A and Grade B raw and pasteurized milk were studied. Laboratory tests used were total counts on old and new standard methods agar; count on blood agar; microscopic count; identification of genera and species, quantitative counts of coliform organisms, clostridia spores, yeasts and molds; and toxicity test on guinea pigs.

Agar plate counts of Grade A milk by Standard Methods gave monthly median values ranging from 22,000 to 300,000 for the raw, and from 450 to 2,400 for the pasteurized product. The range of Grade B milk, by the same methods, was from 160,000 to 890,000 for the raw, and 8,400 to 21,000 for the pasteurized. Use of the newly adopted official agar raised the counts of most samples of milk and increased considerably the number of samples of Grade B milk with counts in excess of the limit allowed by the present Sanitary Code of New York City. From this and other tests, the conclusion is made that Grade A milk is a more uniform and cleaner product than Grade B milk.

M.W.Y.

**617. The Responsibility of The Milk Producer to the Consumer.** W. E. KRAUSS, Ohio Agr. Exp. Sta., Wooster, Ohio. The Bimonthly Bull., 25: 203, p. 31. March-Apr., 1940.

In line with producing clean, safe milk, it is the responsibility of the producer to actively participate in movements to eradicate from herds diseases which can be transmitted through milk. A second responsibility is to make efforts to lower costs of production while still maintaining high standards. In this the producer can be aided by membership in a dairy herd improvement association. Another duty of the producer is to make use of the latest knowledge of the relationship between good feed and high quality milk, from a nutritional standpoint.

P.H.T.

**618. Essentials for Producing Good Milk and Cream.** D. R. THEOPHILUS, Univ. of Idaho., College of Agr., Moscow, Idaho. Ext. Circ., No. 66. May, 1940.

Simple procedures for producing good milk and cream are listed and discussed. Next in importance to having healthy cows is cleanliness of barns, milkers and utensils. Satisfactory methods of cleaning and sterilizing are given. A diagram of a well-planned milk-house is shown as are photographs of proper equipment.

P.H.T.

**619. Variations in the Percentage of Fat in Cows' Milk.** I. E. PARKIN, Pa. State College, School of Agr. and Exp. Sta., State College, Pa., Circ., 222. May, 1940.

In this circular various reasons are given for the fluctuation in the percentage of fat in cows' milk, such as the breed of cows, inherited characteristics of the individual, seasonal variations, effect of time of freshening, and effect of feed and exercise. Mention is made of experimental work being done with drugs and hormones in an effort to increase fat percentages.

P.H.T.

**620. Watery Appearance of Frozen Homogenized Milk.** G. M. TROUT, Michigan State College, Agr. Exp. Sta., East Lansing, Mich. The Quarterly Bull., p. 10. Aug., 1940.

Experiments were made which showed that homogenized milk freezes slightly more quickly than unhomogenized milk. After the frozen homogenized milk was allowed to melt at 70° F. the milk became snowy or watery. After mixing, however, it appeared as smooth as the unfrozen milk. In general, the higher processing pressures resulted in a more watery appearance when the milk was frozen. The fat of slowly-thawed frozen homogenized milk exhibited marked settling, while that of the rapidly-thawed milk showed the tendency to a lesser extent. For this reason fat analyses of the upper portion of slightly frozen homogenized milk may not give an accurate test of the fat percentage.

P.H.T.

**621. Is One Grade of Milk Desirable?** Editorial, Am. J. Pub. Health, 30: 1111-1112. 1940.

The new regulations for milk in New York City are discussed. On September 1, 1940, the former Grade A and Grade B pasteurized milks were superseded by one grade of pasteurized milk designated as "Approved" with intermediate standards. Certified milk may still be sold.

Bacteriological requirements for the new milk supply before pasteurization are 150,000 per cc. in the country and 400,000 per cc. in the city, in contrast to 100,000 per cc. and 200,000 per cc., respectively, for Grade A. After pasteurization, the standard is the same, 30,000 bacteria per cc. The cooling temperature for the "Approved" milk is 60° F. as against 50° F. for the former Grade A, and the age limit for the sale of the new grade is 48 hours, instead of the 36 hours for Grade A. A cover cap is required for the new grade. The minimum butter fat is set at 3.3 per cent. Whether the new regulations for milk in New York City are an improvement or not is a matter that has aroused much controversy.

M.W.Y.

**622. Automatic Control of Pasteurization. Advantages and Safeguards.** A. W. FUCHS, U. S. Public Health Service, Washington, D. C. Am. J. Pub. Health, 30: 477-482. 1940.

Safeguards of design and operation that must be required to insure proper pasteurization in automatic equipment are described and discussed.

Experience with the phosphatase test has indicated the superior reliability of automatic over manual pasteurization. While the safeguards that have been incorporated in automatic pasteurizers are still imperfect, they have been recently improved so as to eliminate many of their weaknesses; on the other hand, the faults inherent in the human element of manual pasteurization are still present.

M.W.Y.

- 623. A Study of Concentration and Freezing as a Means of Preserving Fluid Whole Milk.** R. T. CORLEY AND F. J. DOAN. *Food Res.*, 5: 369. 1940.

Pasteurization temperature of 82.2° C. (180° F.) were found preferable to lower temperatures. Homogenization after condensation retarded the development of tallowy flavor and coagulation during storage as did the higher pasteurization temperature. Such milk could be stored frozen for fifteen weeks and be reconstituted to normal milk showing *in vitro* digestion characteristics superior to boiled milk and somewhat similar to evaporated and acidified milk. Milk contaminated with copper develops tallowy flavor unless storage period is less than five weeks.

P.A.D.

- 624. High Temperature Short Time Pasteurization.** H. D. KAY. *Milk Industry*, 21: 2. Aug., 1940.

The author discusses the history of the high temperature short time pasteurization in Great Britain. The results of several installments of short time high temperature pasteurizers operating at different temperatures and for different periods of exposure are given. At the present time high temperature short time pasteurization does not meet the legal requirements of pasteurization in England.

W.J.C.

## PHYSIOLOGY •

- 625. Studies on the Secretion of Milk Fat. 3. The Effect of Thyroxine Administration on the Blood Lipoids and on the Nature of the Milk Fat.** JAMES A. B. SMITH AND NOSHIR N. DASTUR, Hannah Dairy Res. Inst., Kirkhill, Ayr. *Biochem. J.*, 34: 1093. 1940.

The administration of thyroxine to cows markedly increased the yields of milk and milk fat. There was, however, no consistent change in the level of the non-fatty solids of the milk except for a slight decrease in the protein concentration.

There was no alteration of any importance in the actual nature of the milk fat during the period when its yield was enhanced except for a slight temporary change at the beginning of the hyperthyroid period. The concentration of sugar in the plasma was increased by some 10-26 per cent during the period of hormone administration and this was accompanied by

a general decrease of 10-20 per cent in the concentration of the main lipoid constituents.

V.C.S.

## MISCELLANEOUS

**626. Locker Plant Operation.** K. F. WARNER, extension meat specialist, U. S. Dept. of Agr. Ice Cream Field, 36: 3, 12. Sept., 1940.

Ten years ago there were only a few dozen food locker plants in 3 or 4 states, while today there are over 2800 locker plants in 44 states. Over 75 per cent of these plants are operated in connection with some other business, such as an ice cream plant, a creamery or an ice plant.

Lockers of about 250 pounds capacity rent for from \$5.00 to \$15.00 a year, with or without additional service and service charges for preparing, packaging and freezing foods.

It is claimed that locker plants can operate successfully with from 100 to 150 lockers if operated in connection with some other business but where plants operate independently 300 to 600 or more lockers are customary. Construction costs vary from \$25.00 to \$40.00 per locker.

The author points out that many farm families are able to produce their own food but they are not equipped to preserve it, thus frozen food lockers make it possible to supplement the ordinary diet of canned and salted foods with fresh home-grown beef steaks during the summer and fresh frozen chicken and strawberries in the winter. Many city families also find uses for frozen food lockers, but families who produce most of their food are better potential customers than families accustomed to buying most of it.

The following disadvantages of locker service are listed: (1) Need of cash outlay when the locker is rented and the food purchased, (2) Foods may deteriorate in these lockers unless properly handled, and (3) There is no delivery service.

The importance of properly packaging and freezing suitable quality products is emphasized and the necessity of proper construction and insulation stressed. The question of selecting the most useful type of locker equipment also deserves serious consideration. It is finally stated "Locker plants and locker service are merely new efforts in the continuing process of adapting refrigeration to family needs."

W.C.C.



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